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. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

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 and copper compounds, but may not be inclusive of the entire body of literature.

Summaries of human epidemiological and ecological studies are presented in Table 2-4, Table 2-5, Table 2-6, and Table 2-7. For these tables, note that the study quality varies and study limitations are included. 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; animal dermal studies are presented in Table 2-3.

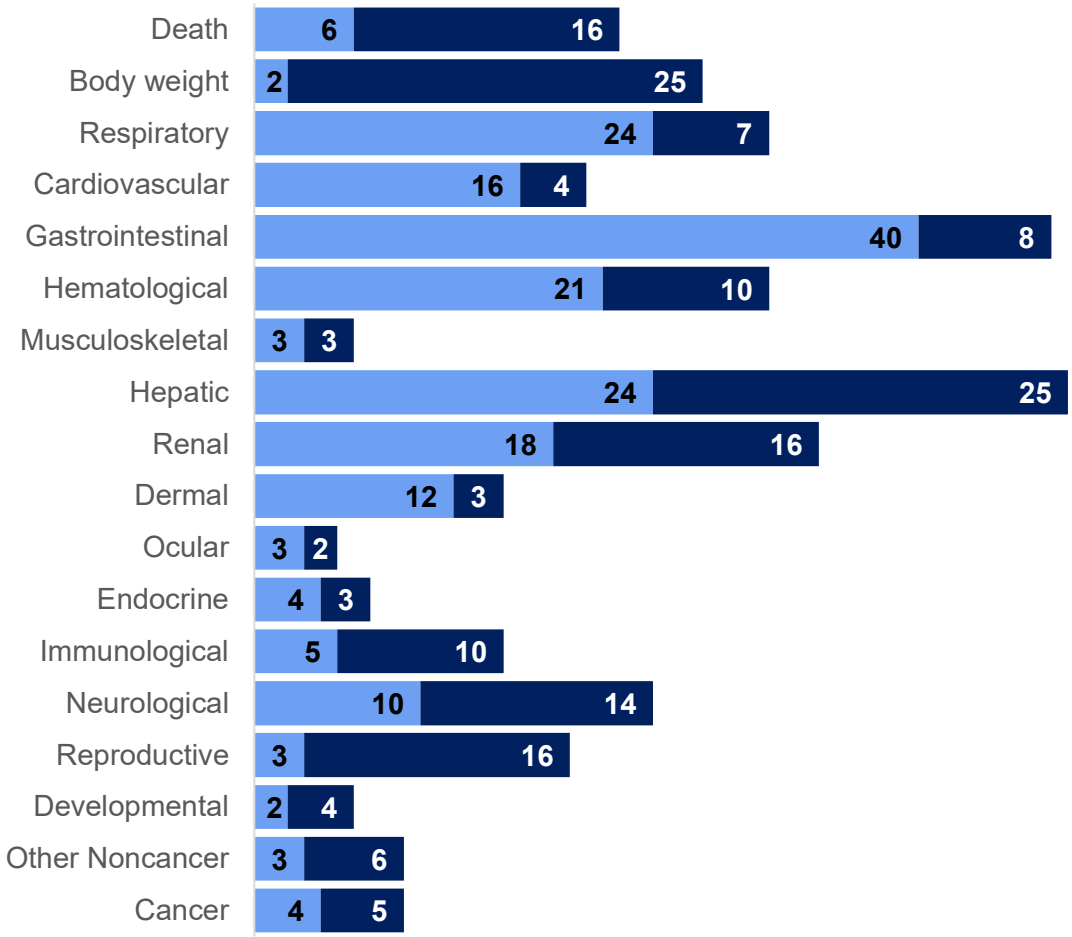
Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be

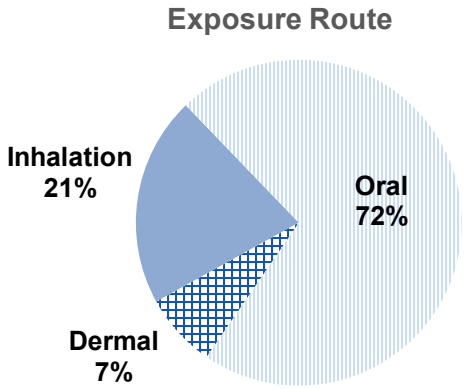
classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints. ATSDR 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.

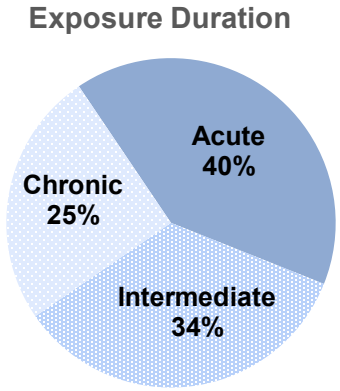
A User's Guide has been provided at the end of this profile (see Appendix D). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.

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 **humans** than **animals** (counts represent studies examining endpoint).







*Includes studies discussed in Chapter 2; studies examined multiple endpoints. A total of 161 studies (including those finding no effect) have examined toxicity.

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Markert et al. 2016									**Copper metal**
1	HUMAN 5M	3 weeks, 3 periods, 6 hours/ period	0, 0.53	HE	Immuno		0.53		Significant increase in C-reactive protein (p=0.001), which increased the greatest between the 6 and 24th hour after the exposure
Rush 1991									**Copper metal**
2	RAT (Sprague-Dawley) 5M, 5F	4 hours (NS)	11.2, 44.8, 88, 208.8	LX	Death			45 F	LC50
								109 M	LC50
Drummond et al. 1986									**Copper sulfate**
3	MOUSE 23-100B	3 hours	0, 0.56, 1.21, 3.3	CS LE OF	Death			0.56	4.2-5.9 day decrease in mean survival and 54-70% increase in mortality
					Resp	3.3			
					Immuno		0.56		Decreased bactericidal activity of pulmonary macrophage
Drummond et al. 1986									**Copper sulfate**
4	MOUSE 15-24B	1-2 weeks 5 days/weeks 3 hours/day	0, 0.12, 0.13	CS LE OF	Death			0.13	25-31% increase in mortality in both sexes and reduced mean survival time by 1.3-1.5 days
					Resp		0.12		Increased alveolar wall thickness and irregular appearance

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Immuno		0.12		Decreased bactericidal activity of pulmonary macrophage
Drummond et al. 1986									**Copper sulfate**
5	HAMSTER 4NS	3 hours	0, 1.21, 3.3	OF	Resp	1.21	3.3		Decreased cilia beating frequency in trachea
Drummond et al. 1986									**Copper sulfate**
6	HAMSTER 4NS	1-2 weeks 5 days/weeks 3 hours/day	0, 0.12, 0.13	HP OF	Resp	0.13			
INTERMEDIATE EXPOSURE									
Johansson et al. 1983									**Copper chloride**
7	RABBIT (NS) 8M	1 month 5 days/week 6 hours/day	0,0.28	OF	Resp	0.28			
					Immuno	0.28			
Johansson et al. 1984									**Copper chloride**
8	RABBIT (NS) 8M	4-6 weeks 5 days/week 6 hours/day	0, 0.28	GN HP	Resp	0.28			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects

[a]The number corresponds to entries in Figure 2-2.

To calculate the copper concentration in studies using copper sulfate, it was assumed that the study used copper sulfate anhydrous (molar mass: 159.61 g/mol) unless otherwise specified that copper sulfate pentahydrate (molar mass: 249.69 g/mol) was used.

B = both sexes; CS = clinical signs; F= female(s); GN = gross necropsy; HE = hematological; HP = histopathology; Immuno = immunological; LE = lethality; LC50 = concentration producing 50% death; LOAEL = lowest-observed-adverse-effect-level; M = male(s); NOAEL = no-observed-adverse-effect-level; NS = not specified; OF = organ function; Resp = respiratory.

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

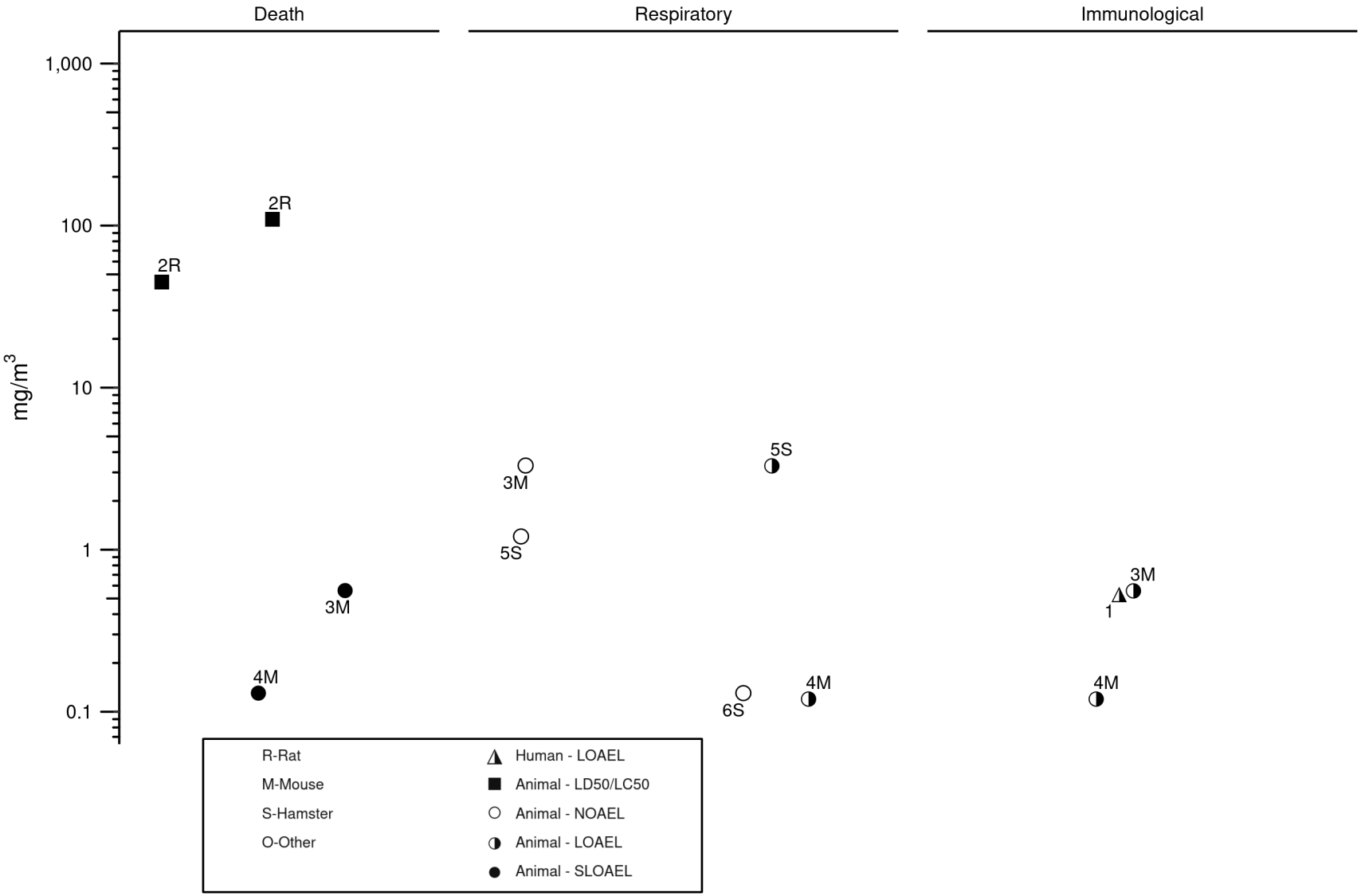


Figure 2-2. Levels of Significant Exposure to Copper – Inhalation
Intermediate (15-364 days)

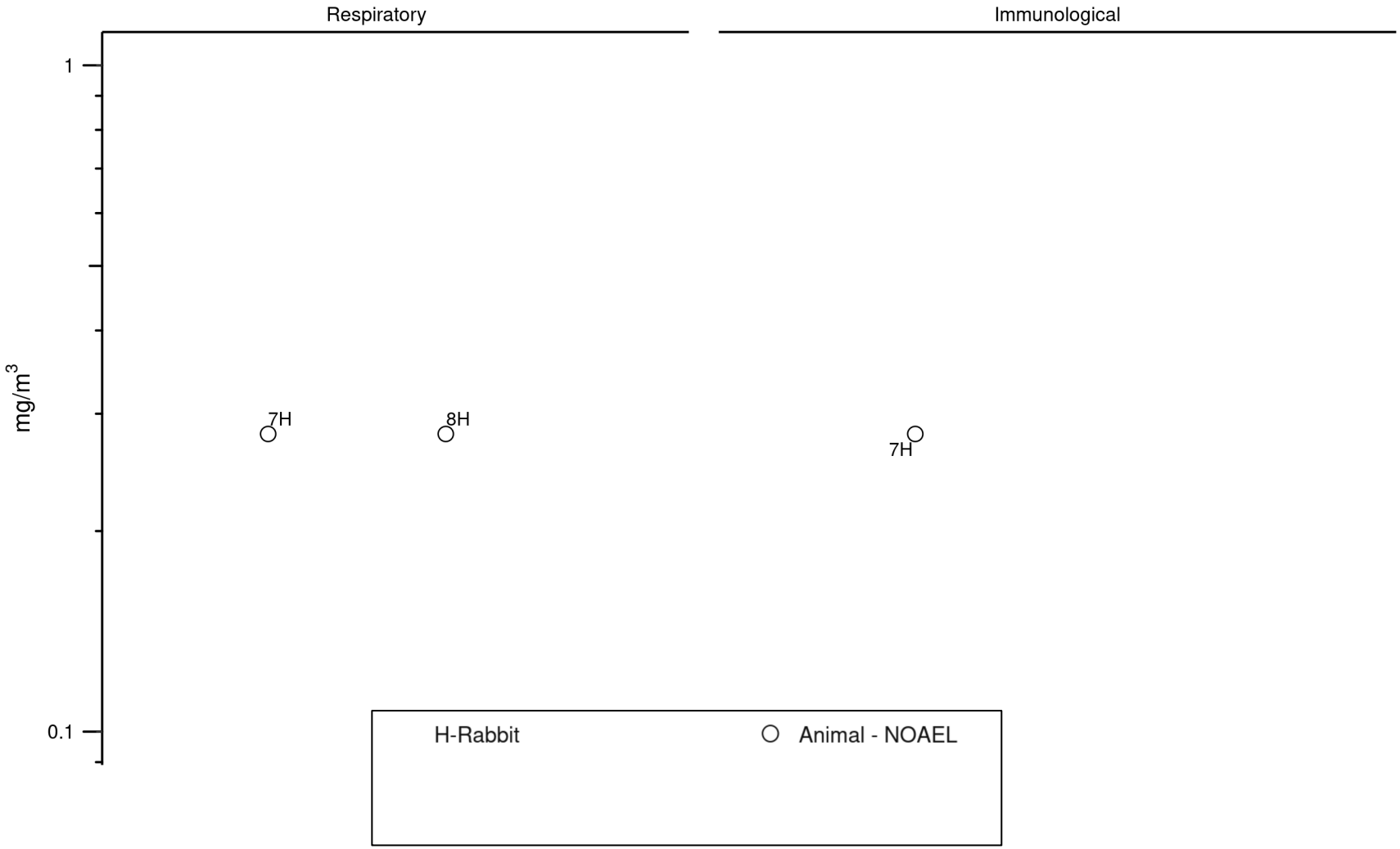


Table 2-2. Levels of Significant Exposure to Copper – Oral (mg/kg/day)

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**									**Copper sulfate**
1	HUMAN 179B	Once (W)	0, 0.006, 0.012, 0.018, 0.025	CS	Gastro	0.01	0.02		Significantly increased frequency of nausea, 17/179 subjects
Araya et al. 2003a**									**Copper sulfate**
2	HUMAN 15M, 15F	Once (W)	0, 0.046	OF	Gastro		0.05		Nausea in 9/30 subjects and delayed gastric emptying
Araya et al. 2003c**									**Copper sulfate**
3	HUMAN 58-73F	Once (W)	0, 0.03, 0.04, 0.06, 0.08, 0.09, 0.12, 0.18	CS WI	Gastro	0.06	0.09		Nausea in 50/269 subjects
Gotteland et al. 2001**									**Copper sulfate**
4	HUMAN 15M, 16F	Once (W)	0, 0.03	CS OF	Gastro		0.03		Nausea (6/31 subjects) and vomiting (2/31 subjects); 36.5% increase in gastric permeability to sucrose
Olivares et al. 2001**									**Copper sulfate**
5	HUMAN 30M, 31F	once (W)	0, 0.006, 0.012, 0.018, 0.025, 0.031, 0.037	CS	Gastro	0.01	0.01		Nausea in 5/53 participants
Pizarro et al. 1999†									**Copper sulfate**
6	HUMAN 60F	2 weeks daily (W)	0.0006, 0.03, 0.07, 0.1	BI BW CS	Bd wt	0.1			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Gastro	0.03	0.07		Abdominal pain, nausea, and/or vomiting; a $BMDL_{10}$ of 0.05 mg/kg/day was calculated for gastrointestinal symptoms ($BMDL_{10}$=0.05 mg/kg/day)
					Hemato	0.1			
					Hepatic	0.1			
					Neuro	0.03	0.07		Increased salivation in six females
Pizarro et al. 2001†									**Copper sulfate and Copper oxide**
7	HUMAN 45F	1 week daily (W)	0, 0.1	BI CS	Gastro		0.1		Nausea, vomiting, and/or abdominal pain
					Hepatic	0.1			
Alharbi et al. 2018									**Copper sulfate**
8	RAT (albino) 10F	7 days daily (IN)	0, 119	BC BI HP	Renal			119	Destroyed glomeruli corpuscles, hyperplasia of the epithelial cells lining the partial layer of Bowman's capsule, and destroyed epithelial lining of the proximal and distal convoluted tubules
Alhusaini et al. 2018a									**Copper sulfate**
9	RAT (Albino) 6M	7 days daily	0, 119	BC BI HP	Hepatic			119	Indications of liver inflammation; elevated hepatic ALT (+299%), NO (+68%), MDA (+44%), and caspase-3 (+>350%); decreased hepatic GSH (55%), SOD (80%) and IL-10 (>45%)

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Alhusaini et al. 2018b									**Copper sulfate**
10	RAT (Albino) 8M	7 days daily	0, 39.8	BI HP OW	Hepatic			39.8	Massive cellular degeneration and hepatocyte necrosis, unspecified reduction in relative liver weight; elevated AST (25%), ALT (>1000%), LDH (>130%), CRP (500%), hepatic MDA (43%), hepatic NO (62%) and protein expression of COX-2 (>500%); significantly lower hepatic GSH (>50%), and SOD (47%)
Rush 1990a									**Copper metal**
11	RAT (Sprague-Dawley) 5M, 5F	Once (NS)	24, 48, 80, 400	LX	Death			37 F	LD50
								42 M	LD50
Yamamoto et al. 2004									**Copper sulfate**
12	RAT (Wistar) NS	once (G)	0, 2.5, 10	CS, FI	Gastro	10			
					Other noncancer	2.5	10		Induced kaolin ingestion behavior (pica behavior)
Babaei et al. 2012									**Copper sulfate**
13	MOUSE (NMRI) 6F	14 days daily (G)	0, 39.8, 79.6	BC HP	Repro			39.8	Decreased number of antral follicles (45%) and ovarian cell damage

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg/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									**Copper sulfate**
14	MOUSE (Swiss albino) 5NS	7 days daily	0, 0.4, 1, 2, 4	BC BI HP	Gastro	2		4	Intestine showing focal ulceration
					Hepatic	2	4		Increased serum PCC and lower cellularity and hemorrhage in liver
					Renal	4			
					Immuno	2	4		Follicular hyperplasia in spleen
					Neuro	4			
Kadammattil et al. 2018									**Copper metal**
15	MOUSE (Swiss albino) 2NS	Once	39.8	LE	Death			39.8	LD50
Kadammattil et al. 2018									**Copper metal**
16	MOUSE (Swiss albino) M NS	Once	0, 4.0	RX	Repro			4	Increased frequency of folded sperm (morphological anomaly)
Kadammattil et al. 2018									**Copper sulfate**
17	MOUSE (Swiss albino) F NS	7 days daily (day 7 to 12 of pregnancy)	0, 4.0	DX RX	Repro	4			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Rush 1990c									**Copper metal**
18	RATS (Sprague-Dawley) 5M, 5F	Once (NS)	40, 60, 80, 400	LX	Death			118 F	LD50
								94 M	LD50
Yamamoto et al. 2004									**Copper sulfate**
19	SHREW (S. murinus) 4F	once (G)	0, 2.5, 31	CS FI	Gastro	2.5	31		15 episodes of emesis in 4/4 animals
					Other noncancer	31			No altered food consumption
INTERMEDIATE EXPOSURE									
Araya et al. 2003b**									**Copper sulfate**
20	HUMAN 327-355B	2 months Daily (W)	0, 0.042, 0.091, 0.17	BC BI CS	Gastro	0.09	0.17		Gastrointestinal symptoms reported by 19.7% of subjects
					Hepatic	0.17			
Araya et al. 2004**									**Copper sulfate**
21	HUMAN 327-355B	2 months daily (W)	0, 0.055, 0.106, 0.169	CS WI	Gastro	0.06	0.11		Gastrointestinal symptoms in 65/355 subjects
O'Connor et al. 2003†									**Copper sulfate**
22	HUMAN 11M, 11F	6 weeks daily (F)	M: 0.018, 0.058 F: 0.017, 0.067	BC BI BW	Hepatic	0.07 F			
						0.06 M			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Olivares et al. 1998									**Copper sulfate**
23	HUMAN 39-50B	9 months daily (W)	0.0378-0.158, 0.0522-0.319	BC BW CS	Bd wt	0.32			
					Gastro	0.32			
					Hepatic	0.32			
Pratt et al. 1985**									**Copper gluconate**
24	HUMAN 3M, 4F	12 weeks (C)	0, 0.15	BC	Gastro	0.15			
					Hemato	0.15			
					Hepatic	0.15			
Turnlund et al. 2004†									**Copper metal**
25	HUMAN 9M	18 days daily (F)	0.02	BC BI IX UR	Immuno	0.02			
Turnlund et al. 2004†									**Copper metal**
26	HUMAN 9M	18 days daily (F)	0.1	BC BI IX UR	Immuno		0.1		Significantly reduced antibody titer against influenza strain compared to controls; 47-fold increase in the antibody in controls, 14-fold in exposed group
Abe et al. 2008									**Copper gluconate**
27	RAT (Fischer-344) 6-8M	6 weeks daily (F)	0, 62	BW HP	Bd wt	62			
					Hepatic	62			

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg/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									**Copper sulfate**
28	RAT (Wistar) 10M	90 days daily (G)	0, 50.9	BI CS HP	Cardio		50.9		33% increase in systolic blood pressure after 3 months
					Repro			50.9	44%, 84%, and 79% reduction in relative testicular weight, serum testosterone level, and serum LH level, respectively; changes of protein expression in testes
Babaei and Abshenas 2013									**Copper sulfate**
29	RAT (Sprague-Dawley) 12M	56 days daily (G)	0, 79.6	HP OW RX	Repro			79.6	13.5% reduction in testicular weight after 56 days and decreased sperm count, percentage of live spermatozoa and sperm motility ($p<0.001$)
Behzadfar et al. 2017									**Copper sulfate**
30	RAT (Wistar) 7M	21 days daily (W)	0, 19.9, 39.8, 79.6	GN HP NX	Neuro	19.9		39.8	Spatial memory impairment as measured by the Morris water maze test; 52% increase in hippocampal mitochondria lipid peroxidation (MDA formation) and 29% decrease in glutathione
DeVries et al. 1986									**Copper sulfate**
31	RAT (Sprague-Dawley) 8F	11 months daily (W)	0, 46	BI	Neuro		46		25% decrease of 3,4-dihydroxyphenylacetic acid levels in corpus striatum

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Epstein et al. 1982									**Copper acetate**
32	RAT (Sprague-Dawley) 8M	90 days daily (W)	0, 8.6	BW WI BC	Bd wt	8.6			
					Hepatic		8.6		>100% increase in aspartate aminotransferase activity
Haddad et al. 1991									**Copper acetate**
33	RAT (Wistar) 20-42F	60-73 days (W)	0, 130	BI BW DX HP RX	Bd wt	130			
					Hepatic		130		Histological changes in liver including degenerated hepatocytes and focal necrosis
					Renal		130		Histological changes in kidney including cloudy swelling in proximal convoluted tubules
					Develop		130		Delayed growth and development
Hashish and Elgaml 2016									**Copper sulfate**
34	RAT (albino) 8F	30 days daily (F)	0, 1.6	BC BI HP	Hepatic		1.6		Acute cell swelling of hepatocytes and karyolysis of nuclei, mild hyperplasia of portal area lining epithelium of bile ducts

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Renal		1.6		Degeneration of renal tissues and degeneration in lining epithelium of some renal tubules; decreased total protein by >32%, increased urea and creatinine by 50% and >65%; decreased renal CAT, SOD and GSH by 24%, >18, >13% and increased renal MDA by 48%
Kalita et al. 2020									**Copper metal**
35	RAT (Wistar) 6M	1 month daily (G)	0, 25.5	BI BW HP NX	Bd wt	25.5			
					Neuro			25.5	Changes in locomotor activity including reduced distance traveled, time moving, grip strength and reduced latency to fall time on the rotarod test and increased time resting; increased expression of GFAP and caspase-3 in corpus striatum indicating apoptosis
Khushboo et al. 2018									**Copper sulfate**
36	RAT (Wistar) 5M	30 days daily (G)	0, 50.9	BI BW FI HP OW RX UR	Bd wt	50.9			
					Cardio			50.9	Flabby, enlarged, and congested heart
					Gastro			50.9	Thickened stomach wall with corrugated mucosa

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic			50.9	Enlargement of liver with dark spots and borders swollen, friable and yellow in color; decreased relative liver weight by 30%
					Renal			50.9	Bilateral enlargement of kidney with a dark brown color; decrease in relative kidney weight by 30% and increased urea (+70%) and creatinine (+67%)
					Dermal		50.9		Rough dried skin with alopecia especially in the abdominal region
					Ocular		50.9		Paleness of mucous membranes of eyes and pads extremities
					Immuno		50.9		Congested and enlarged spleen
					Neuro			50.9	Swollen, congested, and edematous brain; slow activity
					Repro			50.9	>30% increase in sperm head abnormalities and >14% increase in sperm tail abnormalities; degeneration of epididymides, disrupted spermatogenesis, irreversible histological changes in testes, tubular and testicular degeneration; decreased relative weight of testes
					Other noncancer		50.9		Reduced food consumption and water intake by 29% and 41%

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Kumar and Sharma 1987									**Copper sulfate**
37	RAT (Albino) 15M	30 days daily (G)	0, 39.8	BC BI BW	Hemato		39.8		Anemia as evidenced by significant reduction in RBCs (52%) and hemoglobin levels (47%)
					Hepatic		39.8		Increased glucose (18%), cholesterol (34%), bilirubin (66%), serum ALT (308%), and decreased total protein levels (60%)
					Renal		39.8		Increased urea levels (161%) indicating kidney damage
Kumar et al. 2015									**Copper sulfate**
38	RAT (Wistar) 18M	30, 60, or 90 days daily (G)	0, 25.5, 50.9	BC BW NX OF	Bd wt			25.5	21.5% decrease in body weight at 90 days
					Hemato		25.5		Reduced hemoglobin by 9-21% at 30, 60, and 90 days
					Hepatic		25.5		Increased ALT, AST, and bilirubin by 190%, 423% and 488%, respectively at 90 days
					Renal		25.5		Increased BUN and BUN/creatinine ratio of 49% and 67%, respectively after 90 days
					Neuro			25.5	Impaired motor coordination and cognitive function including grip strength, latency to fall time, and attention scores

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Kumar et al. 2016a									**Copper sulfate**
39	RAT (Wistar) 18M	30, 60 or 90 days daily (G)	0, 39.8, 79.6	BI BW HP NX	Bd wt		39.8		Unspecified significant weight loss
					Hepatic			39.8	Hepatocellular degeneration and hemorrhage, massive fatty change and centrilobular necrosis, occasional hepatic cell necrosis
					Renal			39.8	Hemorrhage, inflammatory and cellular damage in kidneys, and degeneration of renal intertubular space and Bowmen's capsule
					Neuro		39.8	79.6	LOAEL: gliosis, pyknotic nuclei, and glial nodule formation SLOAEL: Neuronal loss and depleted myelin
Kumar et al. 2016b									**Copper sulfate**
40	RAT (Wistar) 18M	30, 60 or 90 days daily (G)	0, 39.8, 79.6	BC BW HE HP	Bd wt		39.8		Unspecified significant reduction in body weight
					Hepatic		39.8		Reduced TAC (19-33%) and GSH (14-41%), and increased MDA ($p<0.001$) after 30, 60, 90 days
					Renal		39.8		Reduced TAC (14-26%) and GSH (18-48%), and increased MDA ($p<0.001$) after 30, 60, 90 days

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Neuro			39.8	Changes in grip-strength, rotarod test, and Y-maze test; reduced TAC, GSH, and increased MDA ($p<0.01$)
Kumar et al. 2019									**Copper sulfate**
41	RAT (Sprague-Dawley) 5M	16 weeks daily (G)	0, 4.0, 8.0	BC, BW, CS, NX	Bd wt	8			
					Musc/skel		4		Impaired muscle strength in rotarod test
					Neuro			4	Decreased locomotor activity and neuromuscular coordination, reduced catalase and SOD activity in brain tissues ($p<0.0001$), decreased passive avoidance response, less exploration time
Liu et al. 2016									**Copper sulfate**
42	RAT (Wistar) 10M	30 days daily (G)	0, 39.8, 79.6, 159	HP OW RX	Repro		39.8	79.6	LOAEL: Decreased sperm count (14%); 15% and 13% decrease in LH and FSH SLOAEL: Significant 62% reduction in sperm count and 47% increase in sperm malformation rate; significant reduction in testosterone, FSH and LH, by 43%, 23%, and 21%, respectively

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
NTP 1993									**Copper sulfate**
43	RAT (Fischer-344) 5M,5F	15 days daily (W)	M: 0, 10, 29, 36, 45, 96; F: 0, 10, 26, 31, 71	BW CS GN HP WI	Death			71 F	100% mortality
								45 M	100% mortality
					Bd wt	26 F	31 F		16% weight loss
						36 M			
					Resp	31 F			
						36 M			
					Cardio	31 F			
						36 M			
					Gastro	31 F			
						36 M			
					Hepatic	31 F			
						29 M			
					Renal	31 F			
							10 M		Protein droplets in epithelial cells of proximal tubule
					Endocr	31 F			
						36 M			
					Immuno	31 F			
						36 M			
					Neuro	31 F			
						36 M			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Repro	31 F			
						36 M			
NTP 1993									**Copper sulfate**
44	RAT (Fischer-344) 5M,5F	15 days daily (F)	M: 0, 23, 46, 92, 198, 325; F: 0, 23, 44, 93, 196, 285	BW CS FI GN HP OW WI	Bd wt	285 F			
						325 M			
					Resp	285 F			
						325 M			
					Cardio	285 F			
						325 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
						325 M			
					Hepatic	285 F			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
						92 M	198 M		Minimal to mild mononuclear inflammatory cell infiltrate in 4/5 males
					Renal	285 F			
						46 M	92 M		Increased protein droplets in cortical tubules
					Endocr	285 F			
						325 M			
					Immuno	285 F			
						325 M			
					Neuro	285 F			
						325 M			
					Repro	285 F			
						325 M			
NTP 1993									**Copper sulfate**
45	RAT (Fischer-344) 10M,10F	13 weeks daily (F)	M: 0, 8, 16, 33, 66, 140 F: 0, 9, 17, 34, 68, 134	BC BI CS GN HP OW UR	Bd wt	134 F			
						66 M		140 M	24% decrease in bodyweight by end of experiment
					Resp	134 F			
						140 M			
					Cardio	134 F			
						140 M			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Gastro	17 F	34 F		In 7/10 females, hyperplasia of the 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 the limiting ridge that forms the junction of the forestomach squamous mucosa with the glandular gastric mucosa
					Hemato	134 F			
						33 M	66 M		Decreases in hematocrit, hemoglobin, reticulocytes, mean cell volume, and mean cell hemoglobin levels and increases in platelet levels
					Hepatic	34 F	68 F		Chronic active inflammation in liver of 6/10 females at 68 mg Cu/kg/day
						16 M	33 M		Chronic active inflammation with focal necrosis in 1/10 males; 112% increase in serum alanine aminotransferase
					Renal	9 F	17 F		Increased BUN by 15% and cytoplasmic alteration in kidneys of 1/10 females
						16 M	33 M		Cytoplasmic alteration in kidneys of 3/10 males
					Endocr	134 F			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
						140 M			
					Neuro	68 F	134 F		Gliosis in brain in 10/10 rats
						66 M	140 M		27% increase in relative brain weight
					Repro	68 F		134 F	Chronic active inflammation of clitoral gland and ovarian cysts in 10/10 rats
						66 M	140 M		Significant 27% increase in relative right testis weight
Rana and Kumar 1980									**Copper sulfate**
46	RAT (Albino) 10M	20 days daily (G)	0, 39.8	BC BW CS GN HP	Bd wt			39.8	>28% lower body weight
					Hemato		39.8		Decreased erythrocyte, hemoglobin, and hematocrit levels by 48%, 38%, and 39%, respectively
					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
					Other noncancer		39.8		Change in paw color from pink to white

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg/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. 2012									**Copper sulfate**
47	RAT (Wistar) 20M	8 weeks daily (G)	0, 39.8, 79.6	BC BI HP RX	Hepatic			39.8	Hepatic lesions including cell swelling in hepatocytes, centrilobular hepatocellular necrosis, mild bile retention, multifocal hepatitis and presence of apoptotic bodies
					Renal			39.8	Renal lesions with mild tubular necrosis and hyaline cast formation in renal tubules
					Repro			39.8	54%, 48%, and 60% decrease in sperm concentration, motility, and viability, respectively
Seven et al. 2018									**Copper sulfate**
48	RAT (Sprague-Dawley) 6M	21 days daily (G)	0, 199	BI, BW, HP	Bd wt	199			
					Hepatic			199	Degenerative and necrotic changes in the liver
					Renal			199	Kidneys showed degeneration and necrosis of mostly the proximal and a minority of distal tubules in the cortex
					Immuno		199		Increased serum tumor necrosis factor alpha (TNF-α) level, 1.55 times over control levels
					Other noncancer		199		21% decrease in food consumption

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Tian et al. 2019									**Copper sulfate**
49	RAT (Sprague-Dawley) 10M	30 days daily (W)	0, 79.6	BI BW HP OW	Bd wt		79.6		10-15% decrease in body weight and reduced body weight gain
					Hepatic		79.6		Significantly increased AST and ALT by 67% and 70%, respectively
Babaei et al. 2012									**Copper sulfate**
50	MOUSE (NMRI) 6F	35 days daily (G)	0, 39.8, 79.6 mg/kg/day	BC HP	Repro			39.8	Significant decrease in number of ovarian follicles (>80%) and corpus luteum (88%), and ovarian cell damage
Cheng et al. 2020									**Copper chloride**
51	MOUSE (Kunming) 12F	90 days daily (W)	0, 2.4	BW HP	Bd wt	2.4			
					Gastro			2.4	Increased histological lesions of cecum and rectum including increased thickness of outer muscularis, widened submucosa, and severe atrophy of central lacteal; changes in rectal microbial gut bacteria composition

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Kheirandish et al. 2014									**Copper sulfate**
52	MOUSE (NMRI) 15M	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 Sertoli cell nuclei diameter, and epithelial height and significantly less meiotic index and spermatogenesis
Kvietkauskaite et al. 2004									**Copper sulfate**
53	MOUSE (BALB/c) 10M	19 weeks ad libitum (W)	0, 5.6, 10.7	BC BI BW HE HP OW	Bd wt	5.6	10.7		10.3% reduction in body weight
					Hemato	10.7			
					Hepatic		5.6		13.6% decrease in total liver protein
					Immuno		5.6		Decreased percent of natural killer (CD4*CD8) and suppressor (CD8*CD4) cells and altered immunoregulatory index
Lu et al. 2009									**Copper sulfate**
54	MOUSE (Kunming) 8M	16 weeks daily (W)	0, 0.08	HP, NX	Neuro	0.08			
NTP 1993									**Copper sulfate**
55	MOUSE (B6C3F1) 5M,5F	15 days daily (W)	M: 0, 10, 24, 58, 133, 367; F: 0, 15, 36, 62, 174, 330	BW GN HP WI	Death			62 F	3/5 died
								58 M	1/5 died

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Bd wt	36 F		62 F	27% weight loss in 2/5 mice
						24 M	58 M		16% 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			
NTP 1993									**Copper sulfate**
56	MOUSE (B6C3F1) 5M,5F	15 days daily (F)	M: 0, 43, 92, 197, 294, 717; F: 0, 53, 104, 216, 398, 781	BW CS FI GN HP OW WI	Bd wt	781 F			
						717 M			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Resp	781 F			
						717 M			
					Cardio	781 F			
						717 M			
					Gastro	104 F	216 F		2/5 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		3/5 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
					Hepatic	781 F			
						197 M			
					Renal	781 F			
						717 M			
					Neuro	398 F	781 F		Unspecified significant increase in relative brain weight
						197 M	294 M		Unspecified significant increase in relative brain weight

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
NTP 1993									**Copper sulfate**
57	MOUSE (B6C3F1) 10M,10F	13 weeks daily (F)	M: 0, 44, 97, 187, 398, 815; F: 0, 52, 126, 267, 536, 1058	BW CS FI GN HP OW	Bd wt	267 F	536 F	1,058 F	LOAEL: 13% decrease in bodyweight SLOAEL: 25% decrease in bodyweight
						97 M	187 M	815 M	LOAEL: 11% decrease in bodyweight SLOAEL: 21% decrease in bodyweight
					Resp	1,058 F			
						815 M			
					Cardio	1,058 F			
						815 M			
					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	126 F	267 F		10% increase in relative brain weight
						97 M	187 M		13% increase in relative brain weight

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Repro	536 F	1,058 F		cyst in clitoral gland in 8/10 rats
						97 M	187 M		12% increase in relative right testis weight
Sakhaee et al. 2014									**Copper sulfate**
58	MOUSE (NMRI) 12M	42 days daily (G)	0, 79.6	BC BI GN HP	Hepatic		79.6		AST and ALT significantly elevated ($p<0.05$) 2.8 and 3.6 times greater than controls
					Repro			79.6	Significantly decreased sperm concentration, motility, and viability by 56%, 71%, and 67%, respectively, and degenerative changes of the seminiferous tubules
Sakhaee et al. 2016a									**Copper sulfate**
59	MOUSE NMRI 6M	28 days once every 2 days (GW)	0, 39.8	HP RX	Repro			39.8	60%, 37%, and 41% decrease of sperm count, motility, and vitality, respectively; depletion and vacuolation of seminiferous epithelium
Sakhaee et al. 2016a									**Copper sulfate**
60	MOUSE NMRI 6M	42 days once every 2 days (GW)	0, 39.8	HP RX	Repro			39.8	61%, 39%, and 39% decrease of sperm count, motility, and vitality, respectively; degeneration of the seminiferous tubules

Table 2-2. Levels of Significant Exposure to Copper – Oral (mg/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. 2016b									**Copper sulfate**
61	MOUSE (NMRI) 6M	42 days ad libitum (W)	0, 39.8	HP RX	Repro			39.8	48%, 43% and 43% decrease in sperm count, motility, and viability, respectively, and disorganization and vacuolation of seminiferous epithelium
Wu et al. 2020									**Copper sulfate**
62	MOUSE (ICR) 60NS	42 days daily (G)	0, 4, 8, 16	HP	Hepatic			4	Increased incidence of granular and vacuolar degeneration in hepatocytes and increased rate of hepatic apoptosis
Seffner et al. 1997									**Copper metal**
63	GN PIG (albino) 5-8NS	6 months daily (IN)	<1.04, 6.6 for 4 weeks then 9.6 for	BI BW HE HP OW	Bd wt	9.6			
					Hepatic	9.6			
					Renal	9.6			
					Endocr	9.6			
					Immuno	9.6			
					Neuro	9.6			
Munley 2003a									**Copper hydroxide**
64	RABBIT (New Zealand) 5F	23 days daily (G)	0, 7.5, 15, 30	BW FI LX	Death			30	2/5 rabbits
					Bd wt	30			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Munley 2003a									**Copper hydroxide**
65	RABBIT (New Zealand) 5-9F	GD 7-28 daily (G)	BW CS DX FI GN HP RX	0, 7.5, 15, 30	Death			30	2/8 rabbits
					Bd wt	30			
					Resp	30			
					Gastro		7.5		Diarrhea in 1/8 rabbits
					Hepatic	30			
					Renal	15		30	One animal died from hemolytic event causing hemoglobin nephropathy and likely renal failure; other dead animal had moderately autolyzed small liver
					Endocr	30			
					Immuno	30			
					Repro	15		30	Slight increase in fetal resorptions, total mean per litter of 1.3 resorptions compared to 0.3 resorptions in controls
					Other noncancer	7.5	15		22% reduction in mean food consumption
Munley 2003a									**Copper hydroxide**
66	RABBIT (New Zealand) 29-64 M,F	GD 7-28 daily (G)	0, 7.5, 15, 30	DX GN	Develop	15		30	12% reduction in mean fetal weights; 4/29 rabbits with omphalocele (protrusion of intestines at the umbilicus)

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Munley 2003b									**Copper hydroxide**
67	RABBIT (New Zealand) 21-22F	GD 7-28 daily (G)	0, 6, 9, 18	BW CS FI GN LX OW RX	Death			18	3/22 rabbits
					Bd wt	18			
					Resp	9		18	Dark discoloration or mottling of lung tissue in 3/21 rabbits
					Gastro		6	18	LOAEL: Increased incidence of diarrhea (5/22 rabbits) SLOAEL: Stomach hemorrhage and/or ulceration in 3/21 rabbits; diarrhea in 2/21
					Hepatic	9		18	Pale liver in 3/21 rabbits
					Renal	18			
					Neuro	9	18		Weakness preceding death in one animal that died during exposure
					Repro	9		18	2 fetuses aborted on gestation day 22
					Other noncancer	6	9		Significant 17% decrease in food consumption
Munley 2003b									**Copper hydroxide**
68	RABBIT (New Zealand) 126-159 M,F	GD 7-28 daily (G)	0, 6, 9, 18	BW DX	Develop	9		18	Increased incidence of delayed skull ossification occurring in 5 fetuses among 2 litters and incidence of supernumerary ribs (110/126 fetuses); significant incidence of hemivertebra (2 fetuses)

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Shen et al. 2005									**Copper metal**
69	RABBIT (White Chinese Big Ear) 5M	50 days daily (W)	0, 16	BC BW HE	Bd wt	16			
					Hemato		16		Changes in blood composition including unspecified decreases in neutrophils, eosinophils, platelets, monophils, and basophils
					Hepatic		16		Unspecified increase of LDL and decrease of TG and VLDL
Kline et al. 1971									**Copper sulfate**
70	PIG (Hampshire-Yorkshire) 12NS	88 days ad libitum (F)	0.1, 1.7, 2.3, 2.7	BC BW FI HE	Bd wt	1.7	2.3		17% reduction in body weight gain
					Hemato	2.7			
Suttle and Mills 1966a									**Copper carbonate**
71	PIG (NS) 6F	46 days daily (F)	0, 16.5	BC BI BW	Bd wt			16.5	Decreased weight gain by 22%
					Hemato		16.5		28% decrease in hemoglobin levels
					Hepatic		16.5		Jaundice in 2 of 6 animals
Suttle and Mills 1966a									**Copper carbonate**
72	PIG (NS) 6F	49 days daily (F)	0, 18.7	BC BI BW	Bd wt			18.7	Decreased weight gain by 27%

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hemato		18.7		25% decrease in hemoglobin levels measured at 6 weeks, and 12% increase in erythrocyte count
					Hepatic		18.7		Severe transient jaundice in 5 of 6 animals between weeks 3 and 6; aspartate aminotransferase activity elevated by >100%
CHRONIC EXPOSURE									
Araya et al. 2012									**Copper gluconate**
73	MONKEY (Tufted Capuchin) 2M, 2F	3 years daily (F)	0, 0.7 increased to 1.05 over first 2 months	BI BW CS FI HP OF	Bd wt	1.05			
					Hemato		1.05		Significantly lower hemoglobin (6%)
					Hepatic		1.05		300% increase in Ki67 positive cells indicative of tissue proliferation induction after 36 months
					Other noncancer	1.05			
Araya et al. 2012									**Copper gluconate**
74	MONKEY (Tufted Capuchin) 2M, 2F	3 years daily (Milk)	0, 0.49 increased to 0.77 over first 2 months	BI BW CS FI HP OF	Bd wt	0.77			
					Hemato	0.77			
					Hepatic	0.77			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Other noncancer	0.77			
Massie and Aiello 1984									**Copper gluconate**
75	MOUSE (C57BL/6N) 8M	850 d (W)	0, 4.2, 8.5, 42	BW CS	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.
[b]Used to derive a provisional acute oral minimal risk level of 0.02 mg/kg/day; the $BMDL_{10}$ of 0.05 mg/kg/day was divided by an uncertainty factor of 3 (3 for human variability). The acute oral minimal risk level was also adopted as the intermediate oral provisional minimal risk level.

**The previous EPA reference bodyweight for adults of 65kg was used to calculate the dose.
†The bodyweight reported by the study was used to calculate the dose.

To calculate the copper dose in studies using copper sulfate, it was assumed that the study used copper sulfate anhydrous (molar mass: 159.61 g/mol) unless otherwise specified that copper sulfate pentahydrate (molar mass: 249.69 g/mol) was used.

ALT = alanine aminotransferase; ALP = alkaline phosphatase; AST = aspartate aminotransferase; BC = serum (blood) chemistry; Bd wt = body weight; BI = biochemical indices; BUN = blood urea nitrogen; BW = body weight; Cardio = cardiovascular; COX-2 = cyclooxygenase 2; CRP = c-reactive protein; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; F= female(s); (F) = feed; FI = food intake; FSH = follicle stimulating hormone; (G) = gavage – not specified; Gastro = gastrointestinal; GFAP = glial fibrillary acidic protein; GGT = γ-glutamyl transferase; GN = gross necropsy; GSH= glutathione; (GW) = gavage in water vehicle; HE = hematological; Hemato = hematological; HP = histopathology; IL-10 = interleukin-10; Immuno = immunological; (IN) = ingestion; LD50 = dose producing 50% death; LDH = lactate dehydrogenase; LDL = low-density lipoprotein; LE = lethality; LH = luteinizing hormone; LOAEL = lowest-observed-adverse-effect-level; M = male(s); MDA = malondialdehyde; Musc/skel = musculo/skeletal; Neuro = neurological; NO = nitric oxide; NOAEL = no-observed-adverse-effect-level; NS = not specified; NX = neurological function; OF = organ function; OW = organ weight; PCC = protein carbonyl content; RBC = red blood cell(s); Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; SOD = superoxide dismutase; TAC = total antioxidant capacity; TG = teratogenicity; UR = urinalysis; VLDL= very low-density lipoproteins; (W) = water; WI = water intake

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

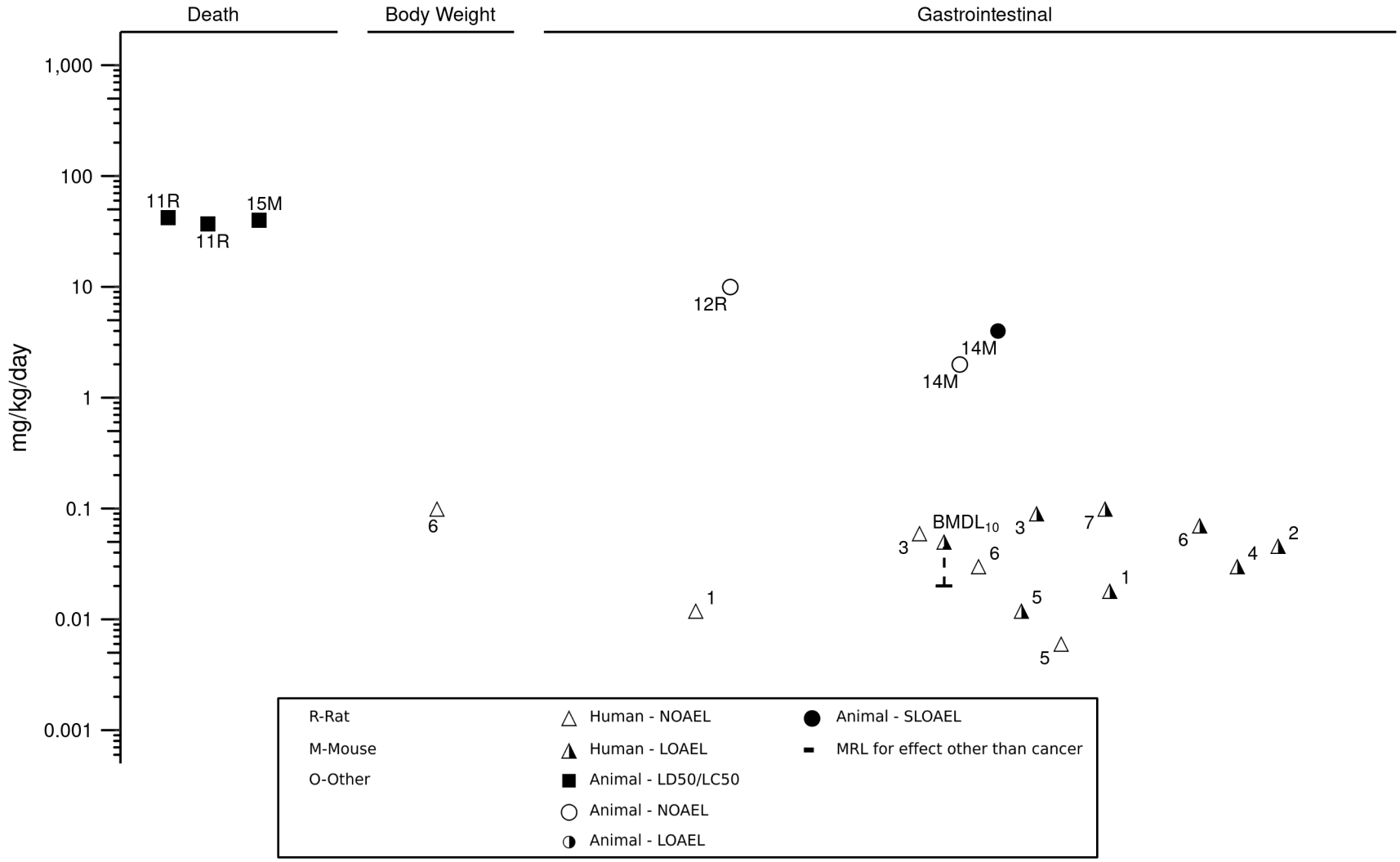


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Acute (≤14 days)

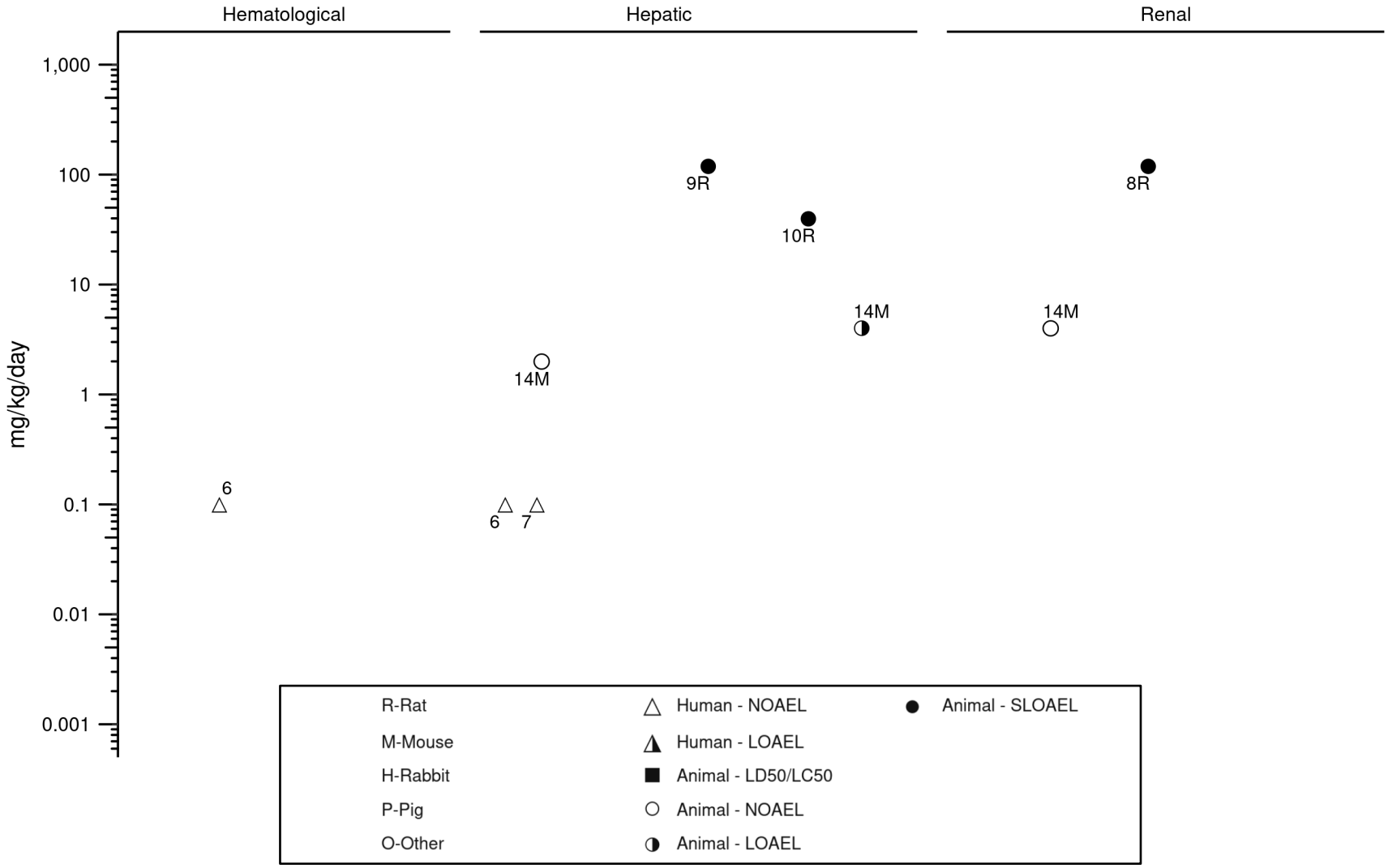


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Acute (≤14 days)

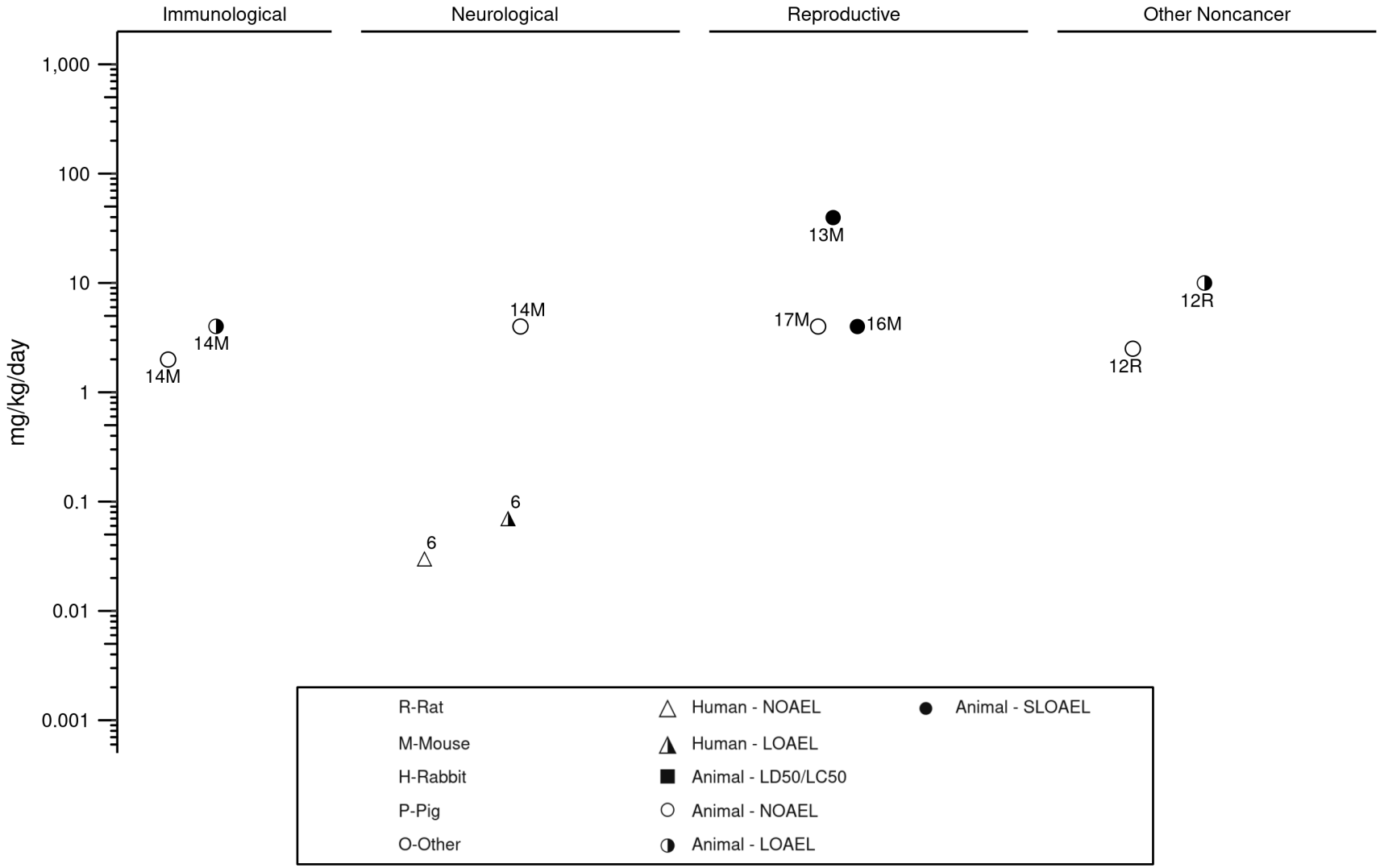


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15-364 days)

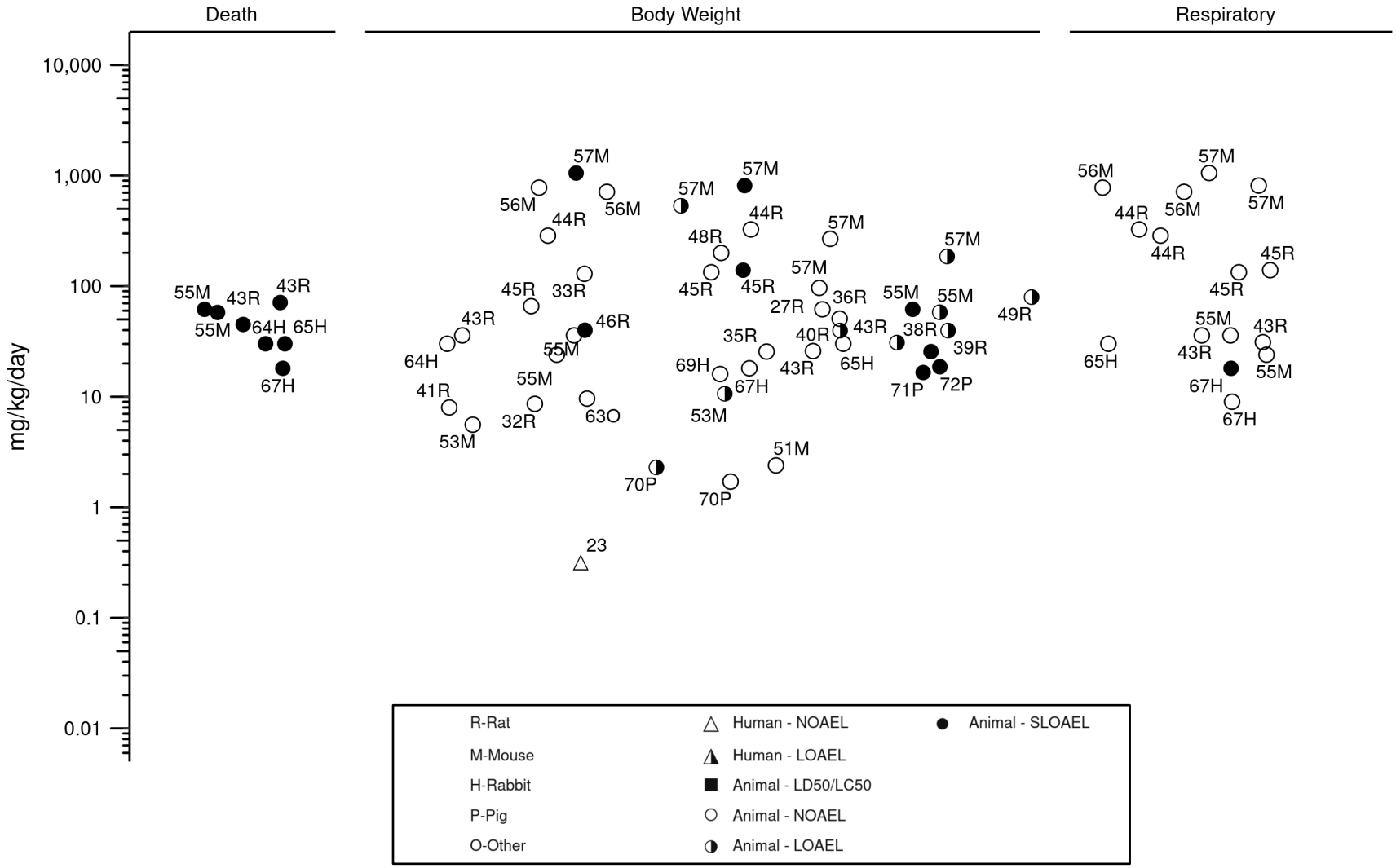


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15-364 days)

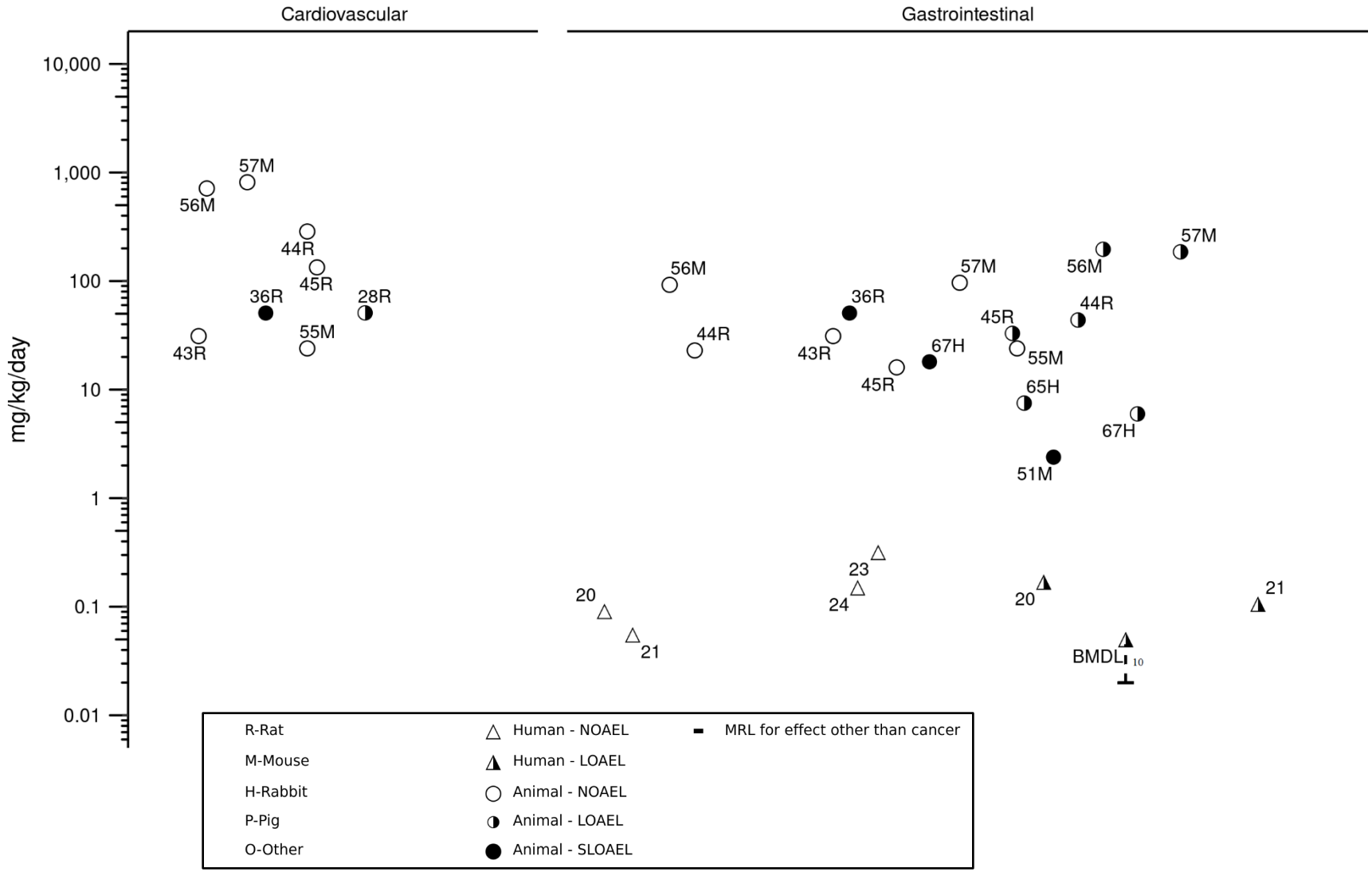


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15-364 days)

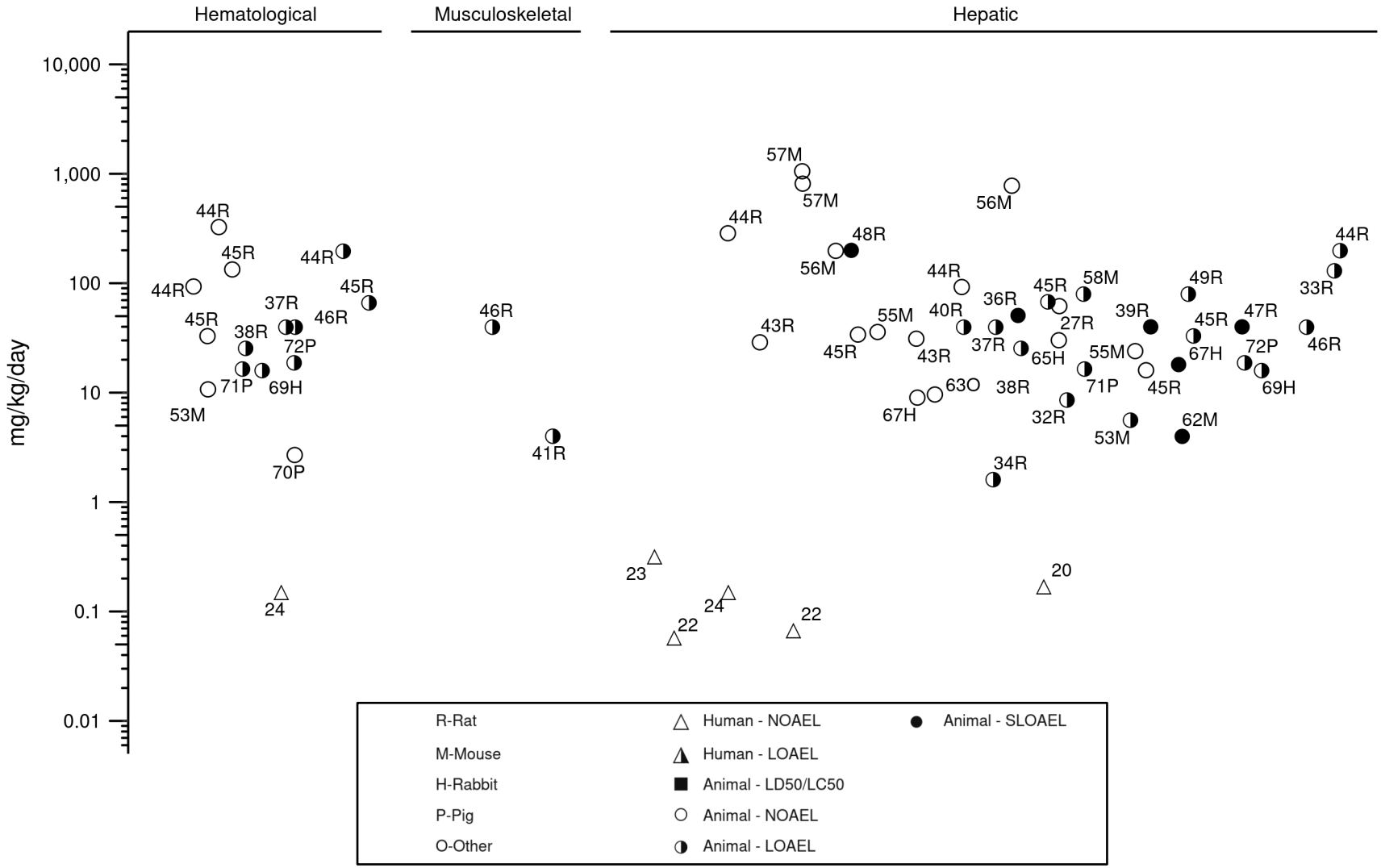


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15-364 days)

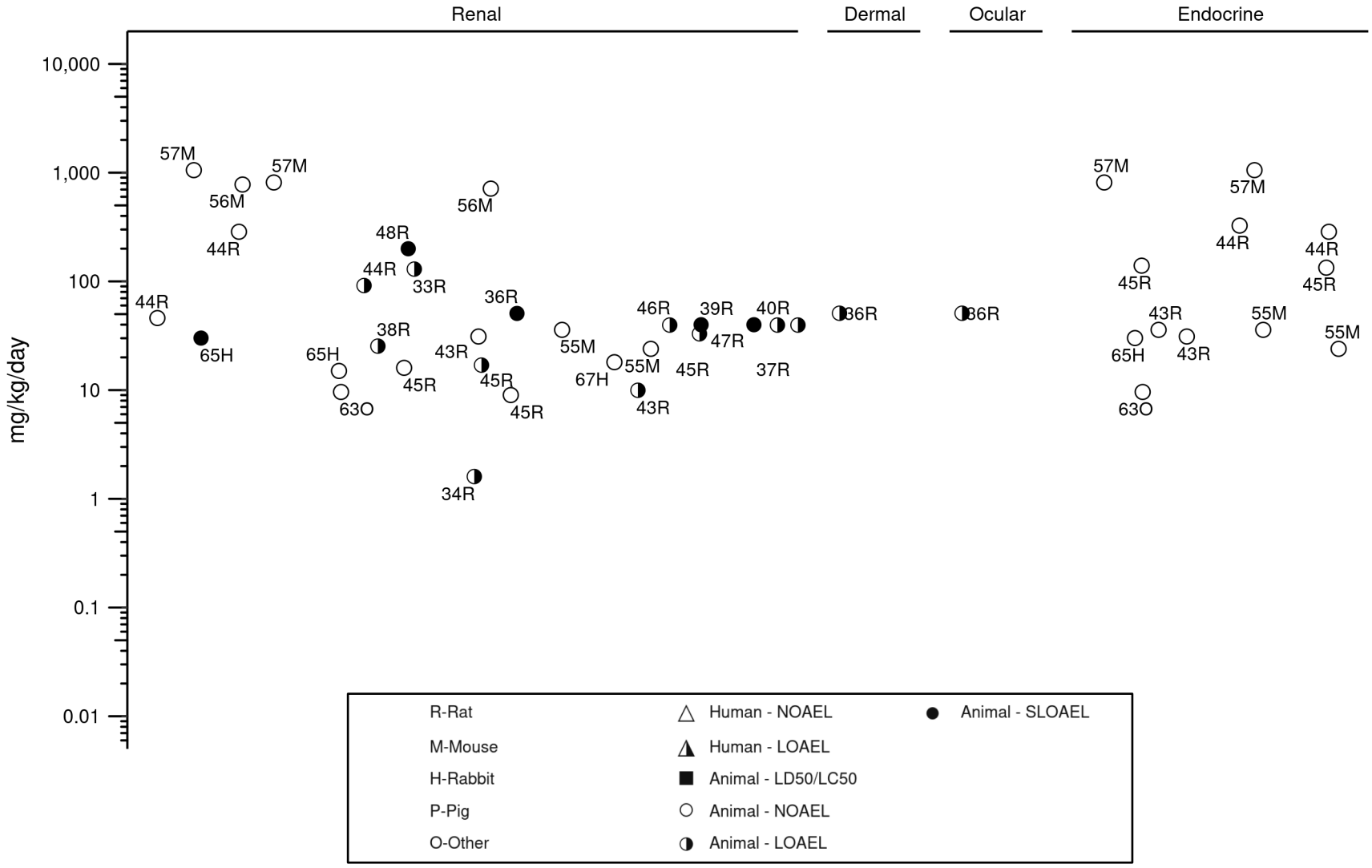


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15-364 days)

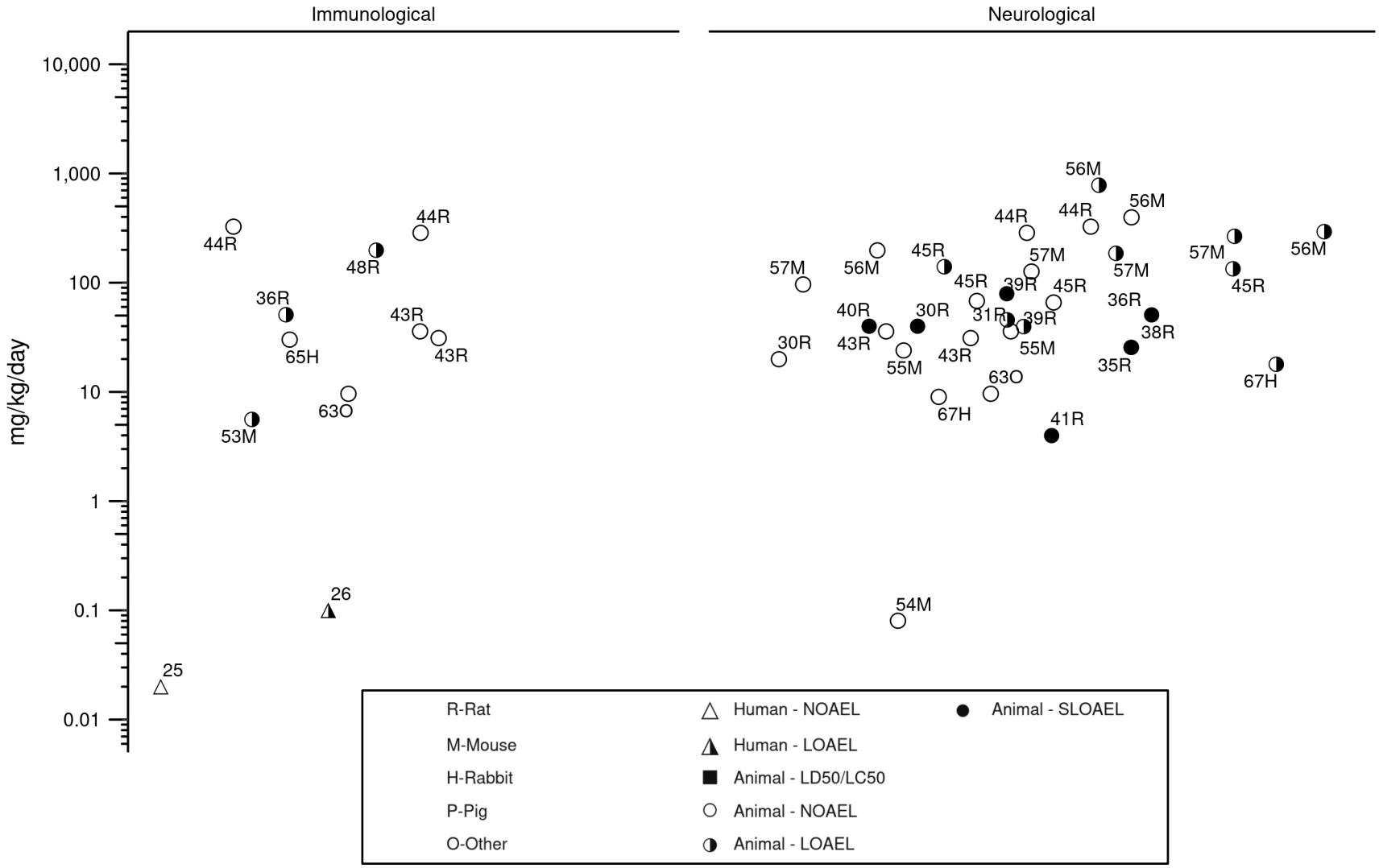


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Intermediate (15-364 days)

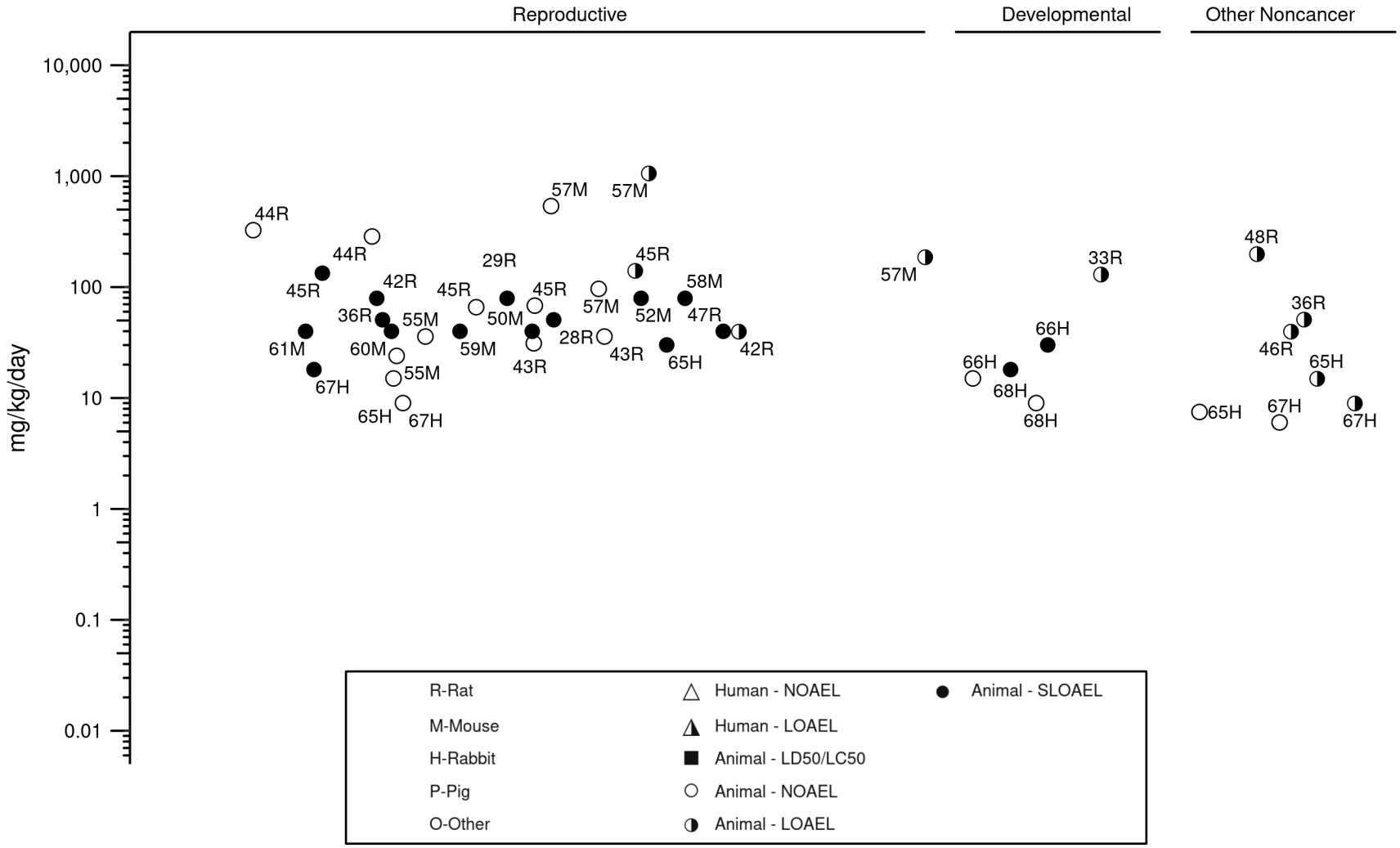


Figure 2-3. Levels of Significant Exposure to Copper – Oral
Chronic (≥365 days)

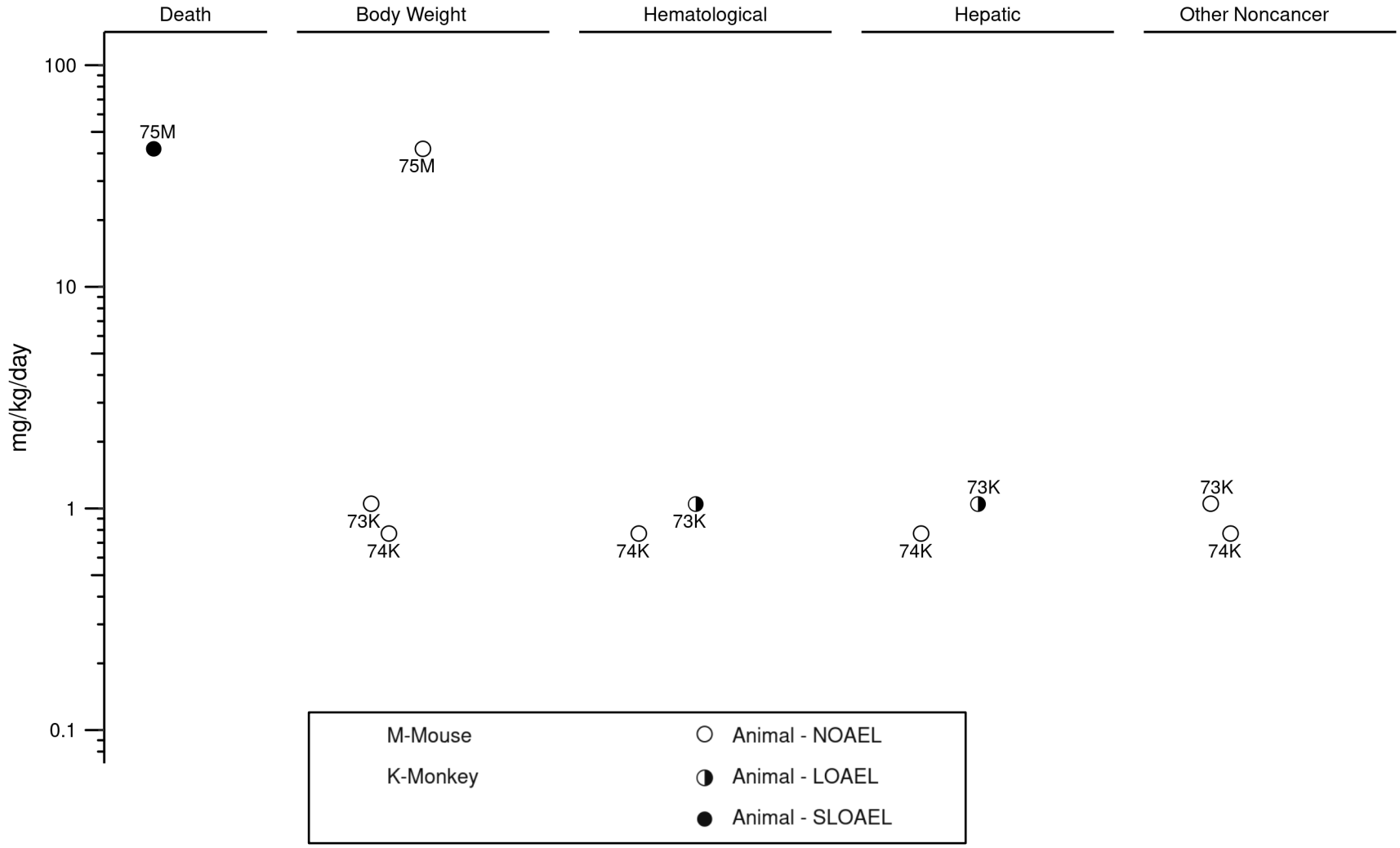


Table 2-3. Levels of Significant Exposure to Copper – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE								
Kuhn 1989b								**Copper oxide**
RABBIT (New Zealand) 5M, 5F	24 hours	1613	LX	Death			1,613	One rabbit died during the exposure period
Rush 1990b								**Copper metal**
RABBIT (New Zealand) 5M, 5F	24 hours	160	LX	Death	160			
INTERMEDIATE EXPOSURE								
Hagemann 1992								**Copper 8-Quinolinolate**
RAT (Albino) 5M, 5F	6 hours/day 5 days/week 4 weeks (NS)	0, 9, 36, 181	BC BW CS FI HE LX OW	Death	181			
				Bd wt	181			
				Resp	181			
				Cardio	181			
				Gastro	181			
				Hemato	181			
				Musc/skel	181			
				Hepatic	181			
				Renal	181			
				Dermal	181			
				Ocular	181			
				Endocr	181			
				Immuno	181 F			

Table 2-3. Levels of Significant Exposure to Copper – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					36 M	181 M		Increased occurrence of necrotic thymic lymphocytes found in higher incidence (5/5 males)
				Neuro	181			
				Repro	181			
				Other noncancer	181			

To calculate the copper concentration in studies using copper sulfate, it was assumed that the study used copper sulfate anhydrous (molar mass: 159.61 g/mol) unless otherwise specified that copper sulfate pentahydrate (molar mass: 249.69 g/mol) was used.

F = female(s); LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = males(s); NOAEL = no-observed-adverse-effect level

Table 2-4. Epidemiological Studies of Humans Exposed to Copper in Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments	Outcomes and Limitations
Multiple Endpoints		
Suciu et al. 1981 **Study Type:** Cohort study of workers who worked in copper sieving operators in 1970 to 1973. The study examined 100, 97, 75, and 97 workers in 1970, 1971, 1972, and 1973, respectively. 51% of workers were 30-39 years of age. A control of 20 healthy non-exposed people were used only as reference for serum copper levels. Medical examinations were performed for the exposure cohort only, and serum copper was measured for both the exposure cohort and controls.	**Exposure:** Inhalation of copper. Copper concentration in air measured. In 1971, concentrations up to 464 mg Cu/m^3 were measured. In 1972, the measured maximum concentration was 132 mg Cu/m^3 and in 1973 it was 111 mg Cu/m^3. Duration of individual exposures varied; most workers were exposed for 6-9 years. Copper serum concentrations were used to classify exposure and workers were divided into 3 groups based on their serum levels, including one "normal" group (normal value of serum copper: 80-120 µg/100 mL serum). **Inclusion/Exclusion Criteria:** Primarily followed-up with workers directly in contact with copper/copper dust. **Covariates Considered/Other Regression Adjustments:** None.	**Outcomes:** Among all years, headaches were the most reported effect, followed by irritability, vertigo, and perspiration. Neurological observances included memory deficits, disturbed concentration, emotional changes and altered motor reactions. Workers examined in 1973 reported paresthesia, spontaneous limb pain, disturbed sensitivity, and disturbances in reflexes. 15.5% of workers who sieved copper presented with emphysematous thorax,5% had bronchial rales, and 18.5% reported dyspnea. 24.5% of workers had functional changes in capillary hypertony. In 1970, 16% of workers had arterial hypertension and by 1973 only 6% had arterial hypertension and palpitations. Workers showed anorexia, nausea, vomiting, hypochondrial pain, abdominal distension, discomfort and sometimes diarrhea. No hepatic cytolysis was observed. Thymol turbidity test showed that 22% of workers had markers for inflammatory conditions. During dermatological examination, 43 workers showed fissured palmo-plantar hyperkeratosis and 44 workers had green impregnation with copper derivatives of the squamous epithelium and of nails. 16% of workers showed sexual impotence. **Limitations:** No control group was used to compare clinical observations. Lifestyle factors not considered, such as smoking, and local exposure

Table 2-4. Epidemiological Studies of Humans Exposed to Copper in Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments	Outcomes and Limitations
		levels. Selection criteria for workers who participated is not stated, thus there is the potential for selection bias.
Death		
Valdes et al. 2012 Ecological study in Santiago, Chile (population 6 million), 1998-2007. National data on daily mortality counts (cause-specific) were assessed.	**Exposure:** Inhalation of copper in ambient air particulate matter. Air pollution data collected from 1998 to 2008 from one air quality monitoring site located in a residential area of Santiago. Median monthly mean copper in $PM_{2.5}$ was 0.75 ng/m^3 (IQR 0.36 ng/m^3). **Inclusion/Exclusion Criteria:** None. **Covariates Considered/Other Regression Adjustments:** Day of the week, long-term time trends, temperature, relative humidity, interaction between $PM_{2.5}$ and monthly averages of mean ratios of copper to $PM_{2.5}$ mass.	**Outcomes:** $PM_{2.5}$ copper concentration was positively associated with cause-specific mortalities, including cardiovascular, respiratory, COPD, and cerebrovascular mortalities. PM-adjusted percent increases in cause-specific mortalities per 10 µg/m^3 increase in 2-day average copper in $PM_{2.5}$ were as follows: cardiovascular: 1.24% (95% CI: 0.44, 2.05); respiratory: 2.09% (95% CI: 0.75, 3.45); COPD: 2.64% (95% CI: 0.32, 5.02); cerebrovascular: 1.44% (95% CI: 0.07, 2.82). **Limitations:** $PM_{2.5}$ measurements were not collected consistently throughout the year, and daily $PM_{2.5}$ data were only available April-September. Averaging elemental concentrations over months may have masked some of the variation. $PM_{2.5}$ data were collected from only one location in the study area and may underestimate pollution as study authors noted this area as “one of the main green areas.” The study only considered short-term impacts of copper exposure via $PM_{2.5}$ inhalation on CVD mortality using day of or 2-day average measurements. Total $PM_{2.5}$ was also associated with each cause-specific mortality endpoint that the authors analyzed and may be a confounder.
Respiratory		
Boogaard et al. 2013 **Study Type:** Prospective cohort study of Dutch residents from 12	**Exposure:** Inhalation of copper in ambient air based on elemental composition of particulate matter. Air monitoring data from 12 locations (8 urban streets and 4 suburban background location) in 2008 and 2010.	**Outcomes:** When adjusted for covariates, reductions in traffic-related air pollutants, such as copper, was associated with a statistically

Table 2-4. Epidemiological Studies of Humans Exposed to Copper in Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments	Outcomes and Limitations
locations. Participants had to be at least 4 years of age. Spirometry tests were only done on participants older than 6 years, and along with airway resistance test these were conducted. Participants also completed questionnaire on respiratory symptoms. Results were compared against pollution data in these areas from 2008 and 2010. 661 participants completed both baseline and follow-up examinations.	Six 1-week samples collected over two 6-month periods in both years. 2008 and 2010 mean copper concentration in air for all 12 sites only differed by 0.01 mg/m^3. Mean copper concentration in air in 2008 for all 12 sites = 29.8 m/m^3. **Inclusion/Exclusion Criteria:** Participants had to be ≥4 years old for airway resistance test, and ≥6 years old for spirometry test. **Covariates Considered/Other Regression Adjustments:** Sex, age, number of cigarette(s)/day, level of education, having cold at time of exam, and any difference in time of year that exams (baseline vs. follow-up) were performed (to account for diurnal fluctuation in respiratory health). In other models, smoking status (yes/never/former), passive smoking, bedroom carpet, animals in home, occupational gas exposure, NO_2 concentrations, and temperature were accounted for. Air samples were measured for and analyses were stratified by PM_{10}, $PM_{2.5}$, soot, NO_x, and Cr and, Fe within PM, in addition to Cu in PM.	significant improvement in forced vital capacity (% change in FVC between 2008 and 2010 = -0.88) **Limitations:** Low response rate of about 10% presents possible selection bias, and there is no information on non-respondents. PM_{10}, $PM_{2.5}$, soot, NO_x, and other elements within PM were not accounted for in the model relating copper to respiratory functions, which could be a source of confounding.
Lavigne et al. 2019 **Study Type:** Ecological study of people living in wards in the London and Oxford area of England (population 13.6 million), 2008-2011. Cardiovascular mortality, respiratory mortality, and lung cancer incidence assessed.	**Exposure:** Inhalation of copper in ambient air particulate matter. Copper in $PM_{2.5}$ and in PM_{10} were obtained from air monitoring data from 20 sites from 2010-2011, and land use regression models were developed to predict $PM_{2.5}$ and PM_{10} elemental composition for the study population. **Inclusion/Exclusion Criteria:** None. **Covariates Considered/Other Regression Adjustments:** Age, sex, ward level tobacco expenditure (pounds/week/inhabitant), % of Asian and White populations at the ward level, the 2007 index of	**Outcomes:** There were 48,483 respiratory deaths that occurred in the study area during the study timeframe. Copper in $PM_{2.5}$ was associated with a slightly increased risk of respiratory mortality (RR = 1.003 per IDR, 95% CI: 0.998, 1.009). Copper in PM_{10} fraction had a very small protective association (RR = 0.988, 95% CI: 0.978, 0.998). Cardiovascular and lung cancer outcomes reported below. **Limitations:** Exposure misclassification is possible due to the ecological study design. The study was

Table 2-4. Epidemiological Studies of Humans Exposed to Copper in Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments	Outcomes and Limitations
	multiple deprivation (IMD) was used as a relative measure of area-level deprivation, which serve as a proxy for socio-economic factors.	reliant on registry data and did not have access to individual-level covariate information other than age and sex. The study did not adjust for co-exposure to multiple pollutants and found that PM components were highly correlated.
Cardiovascular		
Badaloni et al. 2017 **Study Type:** Retrospective cohort study of 1,249,108 residents of Rome. Mortality data was assessed.	**Exposure:** Inhalation of copper in ambient air particulate matter. $PM_{2.5}$ and PM_{10} were measured at 20 sampling sites for 14 days in warm, cold, and intermediate seasons in 2010. Sites were chosen to represent the spatial distribution of residential addresses. Land-use regression models used this data to estimate annual average concentrations of pollutants at the baseline residential addresses of study subjects. Mean copper (± SE) in PM_{10} was 57 ± 26.7 $\mu g/m^3$. Mean (± SE) copper in $PM_{2.5}$ was 15 ± 4.2 $\mu g/m^3$. **Inclusion/Exclusion Criteria:** Subjects were participants in the Rome Longitudinal Study (RoLS) 2001 census administrative cohort, which included all Rome residents aged 30+ years who had lived in Rome for at least 5 years and did not reside in a prison, hospital, or nursing home. Subjects were excluded if exposure data were unavailable. **Covariates Considered/Other Regression Adjustments:** Sex, date of birth, marital status, place of birth, education level, occupation, socioeconomic position of census block, co-pollutants (e.g., NO_2).	**Outcomes:** 59,434 participants died from CVD and 22,234 died from IHD. Copper in $PM_{2.5}$ was associated with increased risk of CVD and IHD in unadjusted and total PM mass-adjusted models (total PM-mass adjusted HR for CVD = 1.04, 95% CI: 1.01, 1.07; PM-adjusted HR for IHD = 1.08, 95% CI: 1.04, 1.13 per 5th–95^{th} percentile range increments). Copper in PM_{10} was also associated with increased risk of CVD and IHD in unadjusted and PM-adjusted models (total PM mass-adjusted HR for CVD = 1.05, 95% CI: 1.02, 1.07; PM mass-adjusted HR for IHD = 1.08, 95% CI: 1.03, 1.13 per 5th–95^{th} percentile range increments). **Limitations:** Exposure misclassification is possible because exposure was measured retroactively (regression models were based on measurements in 2010 and applied to residential addresses dating back to 2001). Residential mobility during follow-up was not accounted for. Individual risk factors, such as smoking, physical activity, and diet, were not accounted for.
Lavigne et al. 2019 **Study Type:** Ecological study of people living in wards in the	**Exposure:** Inhalation of copper in ambient air particulate matter. Copper in $PM_{2.5}$ and PM_{10} was obtained from air monitoring data from 20 sites from 2010-2011, and land use regression models were	**Outcomes:** There were 108,478 CVD deaths in the study area during the study timeframe. Copper in $PM_{2.5}$ was associated with increased risk of death

Table 2-4. Epidemiological Studies of Humans Exposed to Copper in Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments	Outcomes and Limitations
London and Oxford area of England (population 13.6 million), 2008-2011. Cardiovascular mortality, respiratory mortality, and lung cancer incidence assessed.	developed to predict $PM_{2.5}$ and PM_{10} elemental composition for study population. **Inclusion/Exclusion Criteria:** None. **Covariates Considered/Other Regression Adjustments:** Age, sex, tobacco weekly expenditure at the ward level, % of Asian and White populations at the ward level, the 2007 index of multiple deprivation as a relative measure of area-level deprivation	by CVD (RR = 1.005 per IDR, 95% CI: 1.001, 1.009). Respiratory and lung cancer outcomes reported in separate entries. **Limitations:** Exposure misclassification is possible due to the ecological study design. The study was reliant on registry data and did not have access to individual-level covariate information other than age and sex. The study did not adjust for co-exposure to multiple pollutants.
Occelli et al. 2020 Retrospective cohort population-level study with cases with data obtained from the French WHO-MONICA population-based coronary heart disease (CHD) registry for the Lille urban area (population 220,000), 2008-2011.	**Exposure:** Inhalation of copper in ambient air particulate matter (PM_{10}). Air pollution data was obtained using air quality monitoring data from 2009. In addition, bioaccumulation of copper in lichen from 2003-2009 was used as an indicator of historic copper levels in ambient air. Study authors developed a composite environmental score (SEnv) for cumulative exposure to air pollution. Each neighborhood was given a SEnv score. Median copper in lichen was 19.2 µg/g (IQR: 15.2, 27.5). **Inclusion/Exclusion Criteria:** CHD cases where secondary prevention measures (medical intervention) were used were excluded. **Covariates Considered/Other Regression Adjustments:** Age, sex, area-level social deprivation, neighborhood spatial structure.	**Outcomes:** SEnv was positively associated with CHD risk (p=0.0151), and copper concentration in lichen was positively associated with SEnv (SP = 0.47, p<0.0001). Relative risk of CHD was 17% higher in neighborhoods in the highest tertile for SEnv compared to those in the lowest (RR = 1.17, 95% CI: 1.05, 1.31). **Limitations:** Exposure misclassification is likely because exposure was measured at the neighborhood level and did not account for possible commuting. Individual CHD risk factors, such as smoking and diet, were not accounted for. Exposure and outcome information were collected retrospectively and for different time periods (outcome was information collected from 2008-2011 and exposure information was measured in 2009).
Ostro et al. 2008 **Study Type:** Ecological study of residents of 6 California counties, 2000-2003. Obtained data from	**Exposure:** Inhalation of copper in ambient air particulate matter. Daily copper in $PM_{2.5}$ was modeled using time-series regression. Mean copper in $PM_{2.5}$ was 0.007 µg/m^3 (IQR 0.007 µg/m^3).	**Outcomes:** Copper in $PM_{2.5}$ was positively associated with CVD mortality among Hispanic subjects in both the 1 and 3 lag day models. Copper in $PM_{2.5}$ was not associated with any change in CVD mortality among white subjects.

Table 2-4. Epidemiological Studies of Humans Exposed to Copper in Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments	Outcomes and Limitations
the state on the daily counts of cardiovascular mortality from areas of interest.	**Inclusion/Exclusion Criteria:** Counties for which 180+ days of $PM_{2.5}$ monitoring data were available from 2000-2003 were included. **Covariates Considered/Other Regression Adjustments:** Time of year, temperature, humidity, day of the week, number of lag days. Analyses were stratified by subject ethnicity and education level.	Copper in $PM_{2.5}$ was positively associated with CVD mortality among non-HS graduates in the 1 lag day model only. Copper in $PM_{2.5}$ was not associated with any change in CVD mortality among HS graduates. **Limitations:** The study has low statistical power due to the small sample size (~350 observations over 4 years). Because of the ecological study design, exposure and outcome were not linked for individual subjects. The study did not adjust for long-term time trends in copper concentrations in $PM_{2.5}$, so results may be sensitive to the sampling period. The study also did not control for total $PM_{2.5}$ in the association between Copper in $PM_{2.5}$ and mortality, which may be a source of confounding. Covariate information was limited to ethnicity and education level.
Ostro et al. 2015 **Study Type:** Prospective cohort population-level study of 101,884 current and former female teachers and administrators in California, 2001-2007. Participants had been previously mailed questionnaires and state mortality data was reviewed.	**Exposure:** Inhalation of copper in ambient air particulate matter. Copper in $PM_{2.5}$ and ultrafine (UF) particles was modeled for each subject on a monthly basis using the emissions inventory in California from 2001-2007. Mean copper in $PM_{2.5}$ was 0.5 $\mu g/m^3$ and mean copper in UF was 0.03 $\mu g/m^3$. **Inclusion/Exclusion Criteria:** Subjects in the California Teachers Study with follow-up information for years 2001-2007. **Covariates Considered/Other Regression Adjustments:** Age, race, marital status, smoking status, pack-years of smoking, body mass index, lifetime physical activity, alcohol consumption, average daily dietary intake of fat, fiber, and calories, menopausal status, hormone replacement therapy,	**Outcomes:** 1,085/101,884 subjects died from ischemic heart disease (IHD). Copper in $PM_{2.5}$ and UF was positively associated with IHD mortality. The hazard ratio for increase in risk of IHD mortality per 10 $\mu g/m^3$ increase in copper in $PM_{2.5}$ was 1.09 (95% CI: 1.04, 1.15). The hazard ratio for increase in risk of IHD mortality per 10 $\mu g/m^3$ increase in copper in UF was 1.06 (95% CI: 1.03, 1.09). Associations of cardiovascular mortality for copper were reported (HR = 1.03; 95% CI: 1.00, 1.05). **Limitations:** The study has limited generalizability because enrollment was limited to women. Enrolled women all shared the same occupation (schoolteachers or administrators), so the study also cannot be generalized to all women. Incidence of IHD was low in this cohort, so risk estimates may

Table 2-4. Epidemiological Studies of Humans Exposed to Copper in Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments	Outcomes and Limitations
	family history of myocardial infarction and stroke, use of blood pressure medication.	be unstable. Exposure models did not account for possible synergism between co-pollutants or total $PM_{2.5}$.
Wang et al. 2017 **Study Type:** Time-series population-level analysis in Pudong New Area of Shanghai, China (population 2.96 million in 2016), 2014-2016. Daily counts of CVD death from 2014-2016 in the district collected.	**Exposure:** Inhalation of copper in ambient air particulate matter. Daily $PM_{2.5}$ measurements were obtained from the Shanghai Environmental Monitoring Center from 2014-2016. Mean copper in $PM_{2.5}$ was 0.02 ± 0.02 SD μg/m^3. **Inclusion/Exclusion Criteria:** Subjects were permanent residents of the Pudong New Area of Shanghai from 2014-2016. **Covariates Considered/Other Regression Adjustments:** Calendar day, seasonality of CVD mortality, long-term CVD mortality trends, and concentrations of O_3, SO_2, NO_2, and CO in $PM_{2.5}$. $PM_{2.5}$ total mass was controlled for.	**Outcomes:** Previous 2-day $PM_{2.5}$ copper concentration was positively associated with cardiovascular mortality. The percent increase in cardiovascular mortality associated with an IQR increase in $PM_{2.5}$ and copper were 1.53% (95% CI 0.37%, 2.69%) before adjusting for other gaseous pollutants. The association remained positive and significant after adjusting for O_3, SO_2, NO_2, and CO concentrations **Limitations:** Exposure measurement error is likely because exposure data was collected from a fixed-site monitor. The study only considered short term effects of copper in $PM_{2.5}$ on daily CVD mortality.
Neurological		
Pujol et al. 2016 **Study Type:** Cross-sectional study of 2,836 children aged 8-12 years from schools in Barcelona, Spain who completed behavioral testing to test motor function. A subgroup of 236 children had a 3D MRI, functional MRI, and diffusion tensor imaging (DTI) to test brain repercussions.	**Exposure:** Air samples were collected from all schools that participants attended to calculate years air pollution levels. Samples were collected twice during 1-week periods separated by 6 months in warm and cold weather months through 2012 and 2013. Indoor air in the classrooms and outdoor air in the school courtyards was measured. The primary source of copper was road traffic, followed by industrial activity. **Inclusion/Exclusion Criteria:** Children with dental braces. DTI images were excluded when image degradation was detectable.	**Outcomes:** Higher copper exposure was associated with poorer motor performance, which was significant for reaction time (p=0.006; β=2.2). This relationship was observed among the main study group and subgroup. Among the subgroup, copper exposure was associated with a higher proportion of gray matter in the brain tissue (striatum). No other significant alterations were seen on the MRI. In the DTI, copper was associated with increased neural tissue fractional anisotropy.

Table 2-4. Epidemiological Studies of Humans Exposed to Copper in Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments	Outcomes and Limitations
		Limitations: Potential head movement in children during imaging may affect the quality. Self-selection bias as parents opted into the study.
Developmental		
Pedersen et al. 2016 **Study Type:** Retrospective birth cohort study of 34,923 mother-infant pairs from 8 European cohorts, 2008-2011. Birth outcomes obtained from either birth records or from parental reports.	**Exposure:** Inhalation of copper in ambient air particulate matter. PM composition was measured at various European sites from 2008-2013. Mean copper in $PM_{2.5}$ was 3.4 μg/m^3 ± 2.1 SD. Mean copper in PM_{10} was 14.0 μg/m^3 ± 10.6 SD. **Inclusion/Exclusion Criteria:** Data were pooled from 8 European birth cohorts and limited to singleton births from 1994-2008. **Covariates Considered/Other Regression Adjustments:** Maternal alcohol consumption, smoking active and passive smoking during pregnancy, age, pre-pregnancy weight, education level, annual household income, parity, height, infant season of conception, gestational age, sex.	**Outcomes:** Copper in $PM_{2.5}$ and PM_{10} were not associated with mean birth weight in term births (β = 10, 95% CI: -8, 27 and β = 8, 95% CI: -4, 19, respectively). Copper in $PM_{2.5}$ and PM_{10} were not associated with odds of low birth weight (LBW) among term births (OR = 1.08, 95% CI: -0.81, 1.44) and OR = 1.13, 95% CI: -0.92, 1.39, respectively). **Limitations:** Exposure data was collected retrospectively (air quality measurements from 2008-2013 were applied to birth outcomes that occurred from 1994-2008), so temporality cannot be inferred. Subjects' exposure status was determined by home address, and the study did not account for commuting.
Cancer		
Lavigne et al. 2019 **Study Type:** Ecological study of people living in wards in the London and Oxford area of England (population 13.6 million), 2008-2011. Cardiovascular mortality, respiratory mortality, and lung cancer incidence assessed.	**Exposure:** Inhalation of copper in ambient air particulate matter. Copper in $PM_{2.5}$ and PM_{10} was obtained from air monitoring data from 20 sites from 2010-2011, and land use regression models were developed to predict $PM_{2.5}$ and PM_{10} elemental composition for study population. **Inclusion/Exclusion Criteria:** None. **Covariates Considered/Other Regression Adjustments:** Age, sex, tobacco weekly expenditure at the ward level, % of Asian and White populations at	**Outcomes:** There were 24,849 incident cases of lung cancer in the study area during the study timeframe. Copper in $PM_{2.5}$ was associated with increased lung cancer incidence (RR = 1.092 per IDR, 95% CI: 0.943, 1.225). Copper in PM_{10} was associated with increased lung cancer incidence (RR = 0.998 per IDR, 95% CI: 0.912, 1.091). Cardiovascular and respiratory outcomes are reported in separate entries. **Limitations:** Exposure misclassification is possible due to the ecological study design. The study was

Table 2-4. Epidemiological Studies of Humans Exposed to Copper in Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments	Outcomes and Limitations
	the ward level, the 2007 index of multiple deprivation as a relative measure of area-level deprivation	reliant on registry data and did not have access to individual-level covariate information other than age and sex. The study did not adjust for co-exposure to multiple pollutants and found that PM components were highly correlated.
Raaschou-Nielsen et al. 2016 Prospective cohort study of 245,782 people using pooled data from 14 cohorts across 8 European countries, 1997-2008. Incidence of lung cancer analyzed for each cohort the local study centers.	**Exposure:** Inhalation of copper in ambient air particulate matter, both $PM_{2.5}$ and PM_{10}. Air pollution measurements were taken at 250 locations for each cohort from October 2008-May 2011 and land use regression was used to estimate PM composition at subjects' baseline addresses. **Inclusion/Exclusion Criteria:** None. **Covariates Considered/Other Regression Adjustments:** Sex, calendar time, smoking status, smoking intensity, smoking duration, time since quitting smoking, environmental tobacco smoke, occupation, fruit intake, marital status, education level employment status, and area-level employment status. Models were fit with one-pollutant at a time then two-pollutant model fits for each element and concentration of particulate matter, NO_2, and NO_x.	**Outcomes:** There were 1,878 incident cases of lung cancer among study subjects. Copper in $PM_{2.5}$, but not PM_{10}, was associated within increased risk of lung cancer (HR for copper in $PM_{2.5}$: 1.25, 95% CI: 1.01, 1.53, and HR for copper in PM_{10}: 1.14, 95% CI: 0.96, 1.35); however, the effect was somewhat attenuated when controlling for Fe in $PM_{2.5}$. In two-metal models with Fe and Cu, an increase in risk of lung cancer was seen for the main effect of copper in PM2.5 and PM10. **Limitations:** Exposure was assessed retrospectively (air quality data from 2008-2011 was applied to outcome data from 1997-2008). Covariate information was collected only at baseline. Mean subject age ranged from 43-73, so generalizability to younger age groups may not be limited. Use of regression modeling to estimate exposure likely resulted in some exposure misclassification. Because misclassification may be present in varying degrees with each contaminant, when elements are correlated with each other, the misclassification of one element in relation to lung cancer may affect the misclassification present in the other estimate of element and lung cancer risk; this effect may be present in the Cu-Fe relationship explored above.

ARF = acute renal failure, BMI = body mass index, CI = confidence interval, CHD = coronary heart disease, CVD = cardiovascular disease, EAF = electric arc furnace, FEF = mid-expiratory flows, FVC = forced vital capacity; HR = hazard ratio; IDR = interdecile range, IHD = ischemic heart

Table 2-4. Epidemiological Studies of Humans Exposed to Copper in Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments	Outcomes and Limitations

disease, IMD = index of multiple deprivation, MND = motor neuron disease, PM = particulate matter, RR = relative risk, SMR = standardized mortality ratio, UF = ultrafine

Table 2-5. Summary of Health Outcomes in Humans Exposed to Copper in Drinking Water

Reference and study population	Exposure	Cu Dose or Exposure	Outcomes
Gastrointestinal			
Araya et al. 2001 Randomized, prospective, double-blind, controlled study of 179 healthy adults, exposed to copper sulfate added to their drinking water or placebo, after overnight fasting.	**Exposure:** Weekly dose for 5 weeks. Copper sulfate bolus added to drinking water and screened for symptoms for 24 hours after dose. **Adjustments:** none	Copper as copper sulfate added to drinking water: 0, 2, 4, 6, 8 mg Cu/L, and corresponding total copper dose calculated by study were 0.4, 0.8, 1.2, and 1.6 mg Cu, respectively. Total daily doses were 0.006, 0.012, 0.018, and 0.025 mg Cu/kg/day.	Clear dose-response relationship observed with gastrointestinal symptoms and nausea; and nausea and vomiting appeared related. Gastrointestinal (GI) symptoms appeared at 6 mg Cu/L. **Limitations:** Recall bias (self-reporting of symptoms)
Araya et al. 2003a Randomized, double-blind, controlled study of 30 healthy adults, 15 women (mean age 33 years) and 15 men (mean age 37 years), exposed to copper added to their drinking water or placebo, after overnight fasting.	**Exposure:** Each participant underwent two trials receiving, in random order, either placebo or the water-copper sulfate solution and was observed immediately after for 2 hours, then followed up with 24 hours after to check for symptoms. **Adjustments:** none	Copper as copper sulfate added to drinking water: 0 mg Cu/L (placebo), 10 mg Cu/L (0.046 mg Cu/kg).	9 subjects had nausea after receiving copper solution. Presence of copper in the stomach caused delayed gastric emptying presenting as a copper-induced significant delay in decreasing antral area. **Limitations:** Recall bias (self-reporting of symptoms)

Table 2-5. Summary of Health Outcomes in Humans Exposed to Copper in Drinking Water

Reference and study population	Exposure	Cu Dose or Exposure	Outcomes
Araya et al. 2003b Randomized, double-blind community-based study of 1,365 adults in Santiago, Chile where participants were exposed by adding copper to their daily drinking water for 2 months. All participants shared the same water source, and all had copper pipes. Of the participants, 240 participants (60 from each dose group) provided a blood sample. Mean age: 32.9 years ± 12.5. Range of ages: 20-55 years.	**Exposure:** Participants were randomized into 4 groups and added the provided copper solution (as copper sulfate) into 10 liters of drinking water daily to be consumed throughout the day. Subjects reported symptoms daily in a diary and were survey for daily consumption. Acute gastrointestinal symptoms reported in Araya et al. 2004. This study reported results from blood sampling. **Adjustments:** None.	Copper added to drinking water (mg/L): <0.01, 2, 4, or 6. Equating to doses of: 0, 0.042, 0.091, or 0.17 mg Cu/kg/day.	The percentage of participants reporting gastrointestinal symptoms at 6 mg Cu/L (19.7%) was significantly greater compared to controls. Among 240 participants, no exposure-related changes in indicators of copper status and homeostatic regulations, and liver function not affected. **Limitations:** Water consumption and symptoms were self-reported (recall bias)
Araya et al. 2003c Randomized, double-blind, 3x3 factorial (volume x dose) study of 269 adult females (aged 18-60 years) from four different international sites (70 in Santiago, Chile; 68 in North Dakota, U.S.; 58 in Coleraine, Northern Ireland; 73 in Shanghai, China).	**Exposure:** 3 x 3 two-way factorial design had participants given doses of in volumes of either 100,150, or 200 ml bottled drinking water. A control and high dose group also added. Participants fasted the night before visiting the testing center, 3 hours within waking, once a week for 11 successive weeks. Immediately after participants completed a symptoms questionnaire. **Adjustments:** none	Range of copper concentrations, as copper sulfate, among controls, 3x3 factorial and high dose (mg Cu/L): 0, 2, 2.6, 4, 5.3, 5, 8, 12, which equate to doses of 0, 0.03, 0.04, 0.06, 0.08, 0.09, 0.12, 0.18 mg Cu/kg.	Incidence of nausea increased with dose at all study sites and typically within 15 minutes after water consumption. Nausea incidence decreased with time after ingestion. Probability of all gastrointestinal symptoms increased with copper dose, with highest probability of 0.25 (0.2, 0.3) in 1.6 mg Cu group within 15 minutes of ingestion.

Table 2-5. Summary of Health Outcomes in Humans Exposed to Copper in Drinking Water

Reference and study population	Exposure	Cu Dose or Exposure	Outcomes
Araya et al. 2004 Randomized, double-blind community-based study of 1,365 adults in Santiago, Chile where participants were exposed by adding copper to their daily drinking water for 2 months. All participants shared the same water source, and all had copper pipes. Mean participant age: 36.9-38.5 years Sample size: 327-355 adults per exposure group	**Exposure:** Participants were randomized into 4 groups and added provided copper solution into 1 liter of drinking water daily that had to be consumed throughout the day. **Adjustments:** Censored subjects were included and counted per event when their information was incomplete. Data were stratified by time. Covariates included sex, age, total daily fluid volume, volume of water ingested as plain water, and volume of water ingested as mixed fluids.	Copper added to drinking water (mg/L): <0.01, 2, 4, or 6, which equate to doses of 0, 0.055, 0.106, or 0.169 mg Cu/kg/day. End of study concentration in water: 0.05, 2.02, 3.71, or 5.77 mg/L	The risk of gastrointestinal effects increased as copper exposure increased. Women had a higher risk of gastrointestinal symptoms at a lower copper dose than men. At 4 mg/L, women showed an increased risk of gastrointestinal symptoms (RR: 1.53, CI: 1.02, 2.05), while men showed an increased risk of symptoms at 6 mg/L (1.9; CI: 1.02, 2.79). **Limitations:** Water consumption and symptoms were recorded daily by one person in each household, meaning that the data were self-reported, and the timing of exposure compared to symptom onset is unknown.
Buchanan et al. 1999 Retrospective cohort study and nested case-control study in Nebraska. Levels in 1993 had exceeded action level at the time of 1.3 mg/L. Study authors interviewed households who had been exposed to different levels of copper in drinking water. 148 participating households with 451 individuals in cohort study	**Exposure:** Cohort of 182 households were selected from communities where the Nebraska Department of Health had tested the drinking water for copper in 1993. Household members were interviewed about water consumption habits and occurrence of gastrointestinal effects. Water of all participants then measured in August 1994. The cases were identified from these interviews, and then chosen for the nested case-control study,	Households with drinking water copper concentrations >3 mg/L (n = 60), copper concentrations between 2 and 3 mg/L (n = 60), and copper concentrations >1.3 mg/L (n = 62) 1993 Drinking water concentrations (mg/L): ≤1.3, 2.0-2.9, ≥3.0 1994 Drinking water concentrations (mg/L	No significant increase in risk of gastrointestinal symptoms was associated with drinking water copper concentrations greater than 3 mg/L (RR = 1.03; 95% CI: 0.43, 2.49) or 2-3 mg/L (RR = 0.50; 95% CI: 0.18, 1.41) compared to concentrations <1.3 mg/L. **Limitations:** Recall bias (self-reported symptoms)

Table 2-5. Summary of Health Outcomes in Humans Exposed to Copper in Drinking Water

Reference and study population	Exposure	Cu Dose or Exposure	Outcomes
34 individuals in nested-cohort study	with more health interviews and water was tested. **Adjustments:** Risk factors excluded from analysis for affecting <3 participants included medical history of alcoholism, anemia, cancer, high blood pressure, kidney and/or liver problems, and pregnancy; household water system information; previous water contamination with lead or iron; controlled for clustering of people in homes	≤1.3, 1.3-2.9, ≥3.0	
Eife et al. 1999 Retrospective cohort of 29 patients that suffered from nausea, vomiting, colic, and diarrhea (27 from Germany, 1 from Austria, and 1 from USA), 9 adults and 20 children (14/20 children were infants). All patients lived in homes with copper plumbing and reported clinical signs indicating chronic copper intoxication.	**Exposure:** Copper pipes were leaching copper into the water supply. Serum copper ranges in participants by age: 2–7 months: 18.6–35.4 µmol/L 3 years: 61.1 µmol/L 8–16 years: 15.3–24.2 µmol/L “adults”: 13.1–16.4 µmol/L **Adjustments:** none	Concentration in drinking water: 0.1–16.9 mg Cu/L tap water	The authors concluded that the gastrointestinal effects observed in these patients were associated with their copper exposure, along with other systemic diseases of chronic copper intoxication including hepatopathy and natural-killer-cell deficiency. **Limitations:** Study recruitment was inconsistent and involved parent reports, neighbor reports, doctor referrals, and self-reports introducing recall bias. Serum and urine copper levels were only measured in 12/29 patients. Other potential causes of gastrointestinal symptoms do not appear to have been evaluated.
Gotteland et al. 2001	**Exposure:** Controlled exposure of copper added to 200 ml of drinking water, and	Copper added to drinking water (as copper sulfate): 0 mg Cu/L (controls)	There was a copper related increase in gastric permeability to sucrose, but this was not related to gastrointestinal symptoms.

Table 2-5. Summary of Health Outcomes in Humans Exposed to Copper in Drinking Water

Reference and study population	Exposure	Cu Dose or Exposure	Outcomes
Randomized, double-blind study of 31 healthy adults, 15 males, and 16 females (age range: 20-63 years) exposed to copper in drinking water and observed after in a controlled setting for 5 hours and 15 minutes. Participants recorded severity of gastrointestinal symptoms.	then consumption of 200 ml of water with no copper. Equates to a dose of 0.03 mg Cu/kg. **Adjustments:** none	10 mg Cu/L (total copper dose of 2 mg)	After copper ingestion, 22.6% of participants reported gastrointestinal symptoms that were reported as significantly more intense than control participants. **Limitations:** Self-reported perception of symptom intensity
Knobeloch et al. 1994 Study II Between January and June of 1992, a Wisconsin community in newly built homes reported flu-like symptoms attributed to copper-contaminated drinking water tested in the home and distribution system (27 adults and 15 children).	Exposure: Elevated copper in drinking water Adjustments: none	Concentration in drinking water at first draw: 0.16 – 0.65 mg/L	In residents of new homes, Cu concentrations in first draw (rather than flushed) of tap water was associated with an increased relative risk of gastrointestinal upset (RR: 5.25; CI: 1.85, 14.91). Symptoms include diarrhea and abdominal pain. **Limitations:** Recall bias (self-reported symptoms)
Knobeloch et al. 1994 Study III A Wisconsin community was found to have elevated copper levels in their drinking water. 60 residents (ages 15 months to 91 years; mean: 52 years) living in 37 homes responded to health surveys reporting their residence history, age, water	**Exposure:** Elevated copper in drinking water **Adjustments:** none	Concentration in drinking water: 0.09 – 5.3 mg/L	Time of residence at the home (<1 year or >1 year) was associated with an increased relative risk of gastrointestinal symptoms (RR: 8.30, CI: 2.21, 31.09). **Limitations:** Recall bias (self-reported symptoms)

Table 2-5. Summary of Health Outcomes in Humans Exposed to Copper in Drinking Water

Reference and study population	Exposure	Cu Dose or Exposure	Outcomes
consumption habits, and any symptoms.			
Knobeloch et al. 1994 Study IV In 1992, water from water fountain and bathroom sinks in a Wisconsin university building had high levels of copper as water appeared blue in color. Health questionnaires, water consumption, and time in the building was recorded from employees. 41 respondents (17 women, 14 men), age range 27-67 years, mean age: 47.5 years).	**Exposure:** Building water with elevated copper levels **Adjustments:** none	Daily intake from drinking water: 0 – 3.8 mg Cu/day	Daily copper intake of 0.6-3.8 mg Cu/day was associated with increased relative risk of gastrointestinal symptoms (RR: 4.95, CI: 1.56, 15.75), compared to a daily copper intake of 0-0.55 mg Cu/day. Drinking water primarily from the fountain (higher copper concentrations) was associated with increased relative risk of gastrointestinal symptoms (RR: 4.64, CI: 0.66, 32.55) **Limitations:** Recall bias (self-reported symptoms), non-response bias, and small sample size
Knobeloch et al. 1994 Study V Residents of an apartment building in Wisconsin reported bitter-tasting water and asked the local health department to inspect the water supply.19 residents (13 adults and 6 children) filled out health surveys including information on residential history, water use, and health status.	**Exposure:** household tap water with elevated copper levels **Adjustments:** none	Daily intake from drinking water: 0.5 – 8.1 mg Cu/day (mean: 3.4 mg Cu/day)	Relative risk of gastrointestinal upset was higher in children (<18 years) compared to adults (>18 years) (RR: 1.14, CI: 0.73,1.78), but higher copper dose was not associated with higher risk of gastrointestinal upset (RR: 0.74, CI: 0.45, 1.23). **Limitations:** Recall bias (self-reported symptoms) and small sample size

Table 2-5. Summary of Health Outcomes in Humans Exposed to Copper in Drinking Water

Reference and study population	Exposure	Cu Dose or Exposure	Outcomes
Knobeloch et al. 1998 Study 1 Elevated copper levels were found in new or recently remodeled homes of 188 people from 89 families in Wisconsin. Residents were given health surveys to report symptoms and drinking water source.	**Exposure:** household tap water with elevated copper levels **Adjustments:** none	Concentration in drinking water: 0.013 – 4.3 mg/L	A daily intake of >3 mg Cu/day was associated with an increased risk of diarrhea (RR: 1.83, CI: 1.13, 58.36), indigestion (RR: 2.17, CI: 1.09, 4.03), and cramps (RR: 4.98, CI: 2.17, 11.46) when compared to those with <3 mg Cu/day. Symptoms reported include indigestion, diarrhea, abdominal cramps and/or nausea. **Limitations:** symptoms were self-reported via questionnaire
Knobeloch et al. 1998 Study 2 In 1996, residents of a mobile home park in Wisconsin reported “blue water” with a metallic taste and gastrointestinal symptoms. New copper pipes had been added to the water distribution system. 13 families completed health surveys (22 adults and 16 children aged 1-17 years).	**Exposure:** water samples were collected from 10 homes **Adjustments:** age of the home, use of water treatment devices	Concentration in drinking water: 0.4 – 3.7 mg/L	No statistical analysis was done to measure the relationship between copper ingestion and health outcomes. Park residents reported diarrhea (29% of respondents) and upset stomachs (50%). Copper levels were not correlated with age of home, and symptoms rates were higher among children than adults. **Limitations:** Recall bias (symptoms were self-reported via questionnaire) and small sample size
Olivares et al. 2001 61 adult participants from Santiago, Chile (age range: 25 to 60 years) were exposed to added copper in either water or an orange-flavored juice.	**Exposure:** Participants were randomized into 6 groups each week, and then given water or juice with added copper sulfate. Thus, concentrations were tested in almost all participants, resulting in 47-61 adults in each exposure group.	Copper (as copper sulfate) concentration in water or juice: 0, 2, 4, 6, 8, 10, 12 mg Cu/L. Doses in water equate to 0, 0.006, 0.012, 0.018, 0.025, 0.031, 0.037 mg Cu/kg.	Significant increase in incidence of nausea at ≥4 mg/L in water. Significant increase in incidence of vomiting at ≥6 mg/L in water. In juice, threshold for nausea at 8 mg/L. No age or gender related differences observed. **Limitations:** Recall bias (self-reporting of symptoms)

Table 2-5. Summary of Health Outcomes in Humans Exposed to Copper in Drinking Water

Reference and study population	Exposure	Cu Dose or Exposure	Outcomes
Participants came in once a week for 12 exposures.	**Adjustments:** none		
Pettersson et al. 2003 Swedish cohort study from two municipalities with repeatedly high copper concentrations in drinking water. 430 families responded to questionnaires, and 430 young children in the study, aged 9 months to 21 months.	**Exposure:** 4703 samples of tap water from the children's homes were used to measure their exposure to copper. Surveys, a short questionnaire on water consumption habits, and parent-reported symptoms were used to determine gastrointestinal health outcomes. **Logistic regression adjustments**: Ages, number of siblings, family history of allergy, number of daily meals obtained by breastfeeding	Concentration in drinking water: 0.01 to 5 mg/L	No statistically significant increase in diarrhea or vomiting was found for higher copper intake. **Limitations**: The data on the gastrointestinal health outcome was self-reported via survey (recall bias).
Pizarro et al. 1999 Prospective, double-blind, Latin square design study in 60 adult women who were randomly assigned 4 concentrations of copper in their drinking water. Participants were split into 4 groups of 15 people; every participant was exposed to each copper concentration and served as their own control. After each 2-week exposure	**Exposure:** Each group was assigned a different sequence of exposure concentrations. Groups consumed water containing 0, 1, 3, or 5 mg/L ionic copper as copper sulfate pentahydrate for a 2-week period followed by a 1-week rest, then consumed the next concentration in sequence. Each dose was tested in each participant.	Concentrations of 0, 1, 3, or 5 mg/L of copper sulfate pentahydrate equated to daily copper intakes of 0.04 1.74, 4.68, and 7.94 mg Cu/day. Doses calculated using the study reported body weight of 64 kg. Doses were 0.0006, 0.03, 0.07, and 0.1 mg Cu/kg/day.	Twelve subjects reported symptoms of abdominal pain, nausea, and/or vomiting; the incidences were 3/60, 1/60, 10/60, and 9/60 in the 0, 0.03, 0.07, and 0.1 mg Cu/kg/day groups, respectively. Nine subjects reported 12 episodes of diarrhea with or without abdominal pain; no association was found between copper concentration in water and diarrhea. There were no significant differences in serum copper, ceruloplasmin, hemoglobin, or liver enzymes levels between the groups.

Table 2-5. Summary of Health Outcomes in Humans Exposed to Copper in Drinking Water

Reference and study population	Exposure	Cu Dose or Exposure	Outcomes
period, participants reported symptoms and blood was taken at the beginning and towards the end of study.	**Adjustments:** none		**Limitations:** There was no certainty that participants prepared and consumed the water as instructed. Participant consumption of the experimental groups decreased from weeks 1-2 to weeks 11-12, which likely lowered copper exposure and subsequent risk of developing gastrointestinal symptoms. Subjects may have also adapted to high copper concentrations over study period.
Pizarro et al. 2001 Prospective, double-blind study in 45 adult women who worked at home, were neither pregnant or lactating, and lived in Santiago, Chile. Subjects randomized into 3 groups of 15 people, and exposure based on Latin square design. Subjects reported any gastrointestinal symptoms daily on a questionnaire.	**Exposure:** Each group was assigned a different sequence of proportions of copper sulfate to copper oxide (0:5, 1:4, 2:3, 3:2, 5:0 mg/L). Total duration was 9 weeks, five 1-week exposure period with 1-week breaks in between. The total concentration at a given time was 5 mg Cu/L. **Adjustments:** none	Dose of 0.1 mg Cu/kg/day was calculated using 1.2 L of water consumption and body weight of 62.7 kg.	Nine subjects reported diarrhea (with or without abdominal pain and vomiting); seven of the 10 episodes of diarrhea occurred during the first half of the study period and the incidence was not related to ratio of copper sulfate to copper oxide. No time effect was observed for nausea, abdominal pain, and vomiting. Eleven subjects reported abdominal pain, nausea, or vomiting; this incidence is significantly higher than the incidence during the periods the subjects ingested plain tap water. The incidences of gastrointestinal symptoms (excluding diarrhea) for each copper sulfate to copper oxide ratio (0:5, 1:4, 2:3, 3:2, and 5:0) were 5, 3, 3, 2, 6, respectively. No differences in activities of liver enzymes between groups. **Limitations:** Subjects were not asked to record when they consumed the water. Subjects may have consumed the water with meals with may have reduced gastrointestinal symptoms.
Pizarro et al. 2007	**Exposure:** Categorized into 3 groups: group 1 (district with	Dose not reported. Sampled households	The odds of GI symptoms were significantly increased in participants who were younger than

Table 2-5. Summary of Health Outcomes in Humans Exposed to Copper in Drinking Water

Reference and study population	Exposure	Cu Dose or Exposure	Outcomes
Retrospective cohort study of 1,778 families (6,782 people) and their drinking water supplies in Talca, Chile following government reports of gastrointestinal symptoms in the population.	copper-based plumbing where residents reported health effects, n=2,613), group 2 (districts with copper-based plumbing where residents reported no health effects, n=2,515), and group 3 (district with polyvinyl chloride plumbing where residents reported no health effects, n=1,654). **Logistic Regression Adjustments:** dichotomous variables for sex, age (<12 vs ≥12 years), pipe replacement or recent construction, water intake (<0.6 vs ≥0.6 L/d), bottled water consumption (<0.2 vs =0.2 L/d), year home was built (<1996 vs ≥1996), time spent at home (<16 vs ≥16 h/d), and whether participant was the first member of the household to get up in the morning.	showed a mean stagnant water concentration of 0.5 ±0.32 mg Cu/L. Measurement 6 months later showed a mean concentration of 0.57 ±0.36 mg Cu/L.	12 years (OR: 1.468, 95% CI: 1.178,1.832), female (OR: 1.226, 95% CI: 1.010, 1.490), lived in a home built during or after 1966 (OR: 1.279, 95% CI=1.005, 1.626), or who drank less than 200 mL of bottled water per day (OR: 1.668, 95% CI: 1.273, 2.185). Additionally, the odds of GI symptoms were significantly increased in participants living in a home with copper plumbing reporting health complaints compared to participants with copper plumbing reporting no health complaints (OR: 1.589, 95% CI: 1.187, 2.128) or compared to participants with plastic plumbing reporting no health complaints (OR: 1.73, 95% CI: 1.301, 2.301). **Limitations:** The health survey and water sampling were conducted months after the original reports of symptoms. Only a subsample of the homes was selected for Cu tap water concentration measurements. GI symptoms were self-reported, and parents reported symptoms for children younger than 12. Residents of homes without copper pipes drank significantly more tap water. Additionally, residents with copper pipes reported significantly more symptoms unrelated to copper ingestion (ex. allergies, bronchitis, emotional stress) compared to residents without copper pipes.
Hepatic			
Dieter et al. 1999 Retrospective case-control of 103 pediatric patients in	**Exposure:** Patient postal codes were used to collect information from local water suppliers on drinking water	Estimated concentration in drinking water: Low: <1 mg Cu/L Medium: 1-2 mg Cu/L	No statistical analysis was performed on the data. 8/103 cases of ECC (7.8% of patients) occurred in patients with high copper exposure. 5 of those 8 cases occurred in children with high liver

Table 2-5. Summary of Health Outcomes in Humans Exposed to Copper in Drinking Water

Reference and study population	Exposure	Cu Dose or Exposure	Outcomes
Germany with confirmed cases of early childhood cirrhosis (ECC) for the years 1984-1994.	quality. For patients whose homes used a private well, researchers relied on self-reported information from the parents regarding the presence of copper plumbing and the use of tap water. Patients were divided into groups of low, medium, high, or very high copper exposure. Additional information about the patients was identified using the patient records. **Adjustments:** none	High:2-3 mg Cu/L Very High: >3 mg Cu/L	copper and copper plumbing/acid well water. Connection with copper exposure considered probable. **Limitations**: Given the retrospective nature of this study, the temporality of exposure may be inaccurate. Additionally, only 53 parents (51.5%) responded to the request for information about their family's water supply and use. No statistical risk calculation was conducted.
Zietz et al. 2003a A cohort study of infants 8-10 months old living in households with copper plumbing. Water samples were collected from 2,944 houses in Berlin, Germany. Sample were taken and submitted by participants.	**Exposure:** Mean copper concentrations measured in tap water from the home. Families with 0.8 mg Cu/L or more in their water and whose infants ingested tap water were recommended for pediatric exams. Blood copper levels of 79 to 250 ug/dL for infants exposed to elevated copper levels. **Adjustments:** none	Mean copper concentration was 0.44 mg Cu/L and 0.56 mg Cu/L for each of the two composite samples	No signs of liver disease were found in the exposed infants during their pediatric exams. No dose relation between liver serum parameters and copper intake. **Limitations:** Some participants were lost to follow up. Composite copper concentrations limited the ability to draw conclusions about copper dosage.
Zietz et al. 2003b A cohort study of infants up to 12 months old from households	**Exposure:** Copper concentrations measured in household tap water	Concentration in stagnated drinking water: >0.01 – 6.40 mg/L	No signs of liver malfunction were found in the infants.

Table 2-5. Summary of Health Outcomes in Humans Exposed to Copper in Drinking Water

Reference and study population	Exposure	Cu Dose or Exposure	Outcomes
with copper plumbing, from mothers who had given birth in or near Gottingen, Germany. 1,674 households participated, and 172 households were studied more closely, and 14 infants were examined by a pediatrician. Family medical history was obtained.	Blood copper levels of 82 to 220 µg/dL, among 11 infants. **Adjustments:** none	Concentration in daytime samples: >0.01 – 3.00 mg/L	**Limitations:** Pediatrician information and blood samples were only available for a very small subset of infants.
Developmental			
Longerich et al. 1991 Case-control study of 56 women in Newfoundland, Canada who had recently given birth to infants. 28 women (cases) gave birth to infants with neural tube defects (NTD) and 28 women (matched controls) gave birth to infants without NTDs.	**Exposure:** Trace elements, including copper, were measured in the women's drinking water **Adjustments:** A Student's t-test was run to compare the mean copper level in the drinking water of participants, but no adjustments were made during the analysis.	Mean water concentrations: 210 ± 285 ppb (controls), 290 ± 367 ppb (cases)	While the mean copper concentration was higher for cases, no significant difference in average drinking water copper concentration was observed between mothers that gave birth to infants with NTD and mothers that gave birth to infants without NTD. **Limitations**: Drinking water samples were collected once and assumed to be indicative of exposure during pregnancy. Mothers were matched by age of their infants, but no other consideration of potential confounding appears to have been made.

CI = confidence interval, ECC = early childhood cirrhosis, GI = gastrointestinal, NTD = neural tube defects, RR = relative risk

Table 2-6. Copper Exposure from Environmental Media and Health Outcome Associations in Human Studies

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, Covariates Considered and Adjustments:	Outcomes and Limitations
Musculoskeletal		
Yang et al. 2016 **Study Population:** Cross-sectional study of 122 rheumatoid arthritis (RA) patients in a medical center in central Taiwan. Patient age ranged from 16-89 years; 34 males and 88 females.	**Exposure:** Possible ingestion of copper in farm soils. Copper levels in farm soils were obtained from a national database and averaged at the town/precinct level. Each town/precinct received a grade for copper concentration as follows: Grade 1 (most severe): >23.83 mg/kg; Grade 2: 15.43-23.83 mg/kg; Grade 3: 7.03-15.43 mg/kg; Grade 4 (least severe): 0-7.03 mg/kg. Blood copper was also measured in 39 subjects. **Inclusion/Exclusion Criteria:** Subjects had to be formally diagnosed with RA. In the comparison group, gout patients had to have had at least one attack and ankylosing spondylitis (AS) patients were formally diagnosed. **Covariates Considered/Other Regression Adjustments:** In subjects for whom blood copper was measured, multiple regression models were adjusted for age, sex, smoking, hemoglobin level, and an array of other heavy metals.	**Outcomes:** RA patients living in Grade 1 towns had significantly higher mean WBC count than those living in Grade 4 towns and significantly higher mean platelet count and erythrocyte sedimentation rate (ESR) than those living in Grade 2, 3, and 4 towns. RA patients living in Grade 1 towns also had significantly higher mean overall RA disease activity score than those living in Grade 3 and 4 towns. RA patients for whom blood copper was measured had higher blood copper levels than gout patients, AS patients, and steel plant workers of similar backgrounds. Among RA patients, blood copper was significantly positively correlated with white blood count (WBC) count, ESR, platelet count, and rheumatoid factor-IgM. **Limitations:** The cross-sectional study design prevents conclusions about a causal relationship between copper concentration in soil or blood copper and RA outcomes. The study did not account for other known determinants of RA outcomes, including genes, viral infections, and vitamin D deficiency. The small sample size (especially for blood copper measurement) limited the statistical power, possibly attenuating the observed association between copper levels in farm soil and blood copper levels in subjects.
Neurological		
Sánchez-Díaz et al. 2018 **Study Type:** Ecological study using mortality data from the National Statistics Institute of Spain (n=9434), 2007-2016.	**Exposure:** Copper released into river basins at 235 sites in Spain from 2007-2015. Exposure was measured at the municipality level. Exposed municipalities were considered those to be within a 20-km river section downstream of at least one	**Outcomes:** The standardized mortality ratio (SMR) for motor neuron disease (MND) was 12.7% higher in copper-exposed municipalities than in unexposed municipalities (IRR = 1.127, 95% CI: 1.075, 1.182).

Table 2-6. Copper Exposure from Environmental Media and Health Outcome Associations in Human Studies

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, Covariates Considered and Adjustments:	Outcomes and Limitations
	site where a copper release was made into the river during the considered time period. **Inclusion/Exclusion Criteria:** none **Covariates Considered/Other Regression Adjustments:** None.	**Limitations:** Exposure misclassification is likely because of the ecological study design (subjects' exposure status was determined by their municipality of residence). The study did not consider exposure to copper from other sources or demographic information for subjects or municipalities. Possible synergistic or antagonistic effects of mixing metals in water, such as cadmium, on MND mortality was not considered, only the effect of isolated metals.
Shen et al. 2014 **Study Type:** Ecological study in 26 provinces and 3 municipal districts of mainland China, 1991-2000. Mortality data of locations analyzed.	**Exposure:** Copper concentration in soil. Mean copper concentration in the "A" soil horizon 24.04 mg/kg ± 6.18 SD. Mean copper concentration in the "C" soil horizon) was 24.96 mg/kg ± 6.65 SD. Mean copper concentration in the A and C soil horizons combined was 49.00 mg/kg ± 12.44 SD. **Inclusion/Exclusion Criteria:** Subjects had to be age 40 or older. **Covariates Considered/Other Regression Adjustments:** Age, gender.	**Outcomes:** Copper in soil was positively associated with relative risk of Alzheimer's Disease (AD) mortality. Compared to regions where copper in soil was <20 mg/kg, regions with the highest copper soil levels (60-80 mg/kg) had an RR of 2.634 (95% CI: 2.626, 2.642). **Limitations:** Due to the ecological study design, exposure data were collected at the county level, so exposure misclassification is likely. Besides age and gender, the study did not adjust for other genetic and environmental risk factors for AD mortality. The study found a positive correlation between zinc and copper correlations in soil (r=0.699) but did not adjust for it in statistical models.
Cancer		
Whitehead et al. 2015 Case-control study of copper levels in carpet dust and childhood acute lymphoblastic leukemia (ALL). Case data were obtained from participants in the California Childhood Leukemia	**Exposure:** Copper loading in carpet dust (possible ingestion), collected between 2001-2006. Median copper loading in carpet dust was 110 μg/m^2 for cases (IQR: 48-220) and 130 μg/m^2 for controls (IQR: 50-290). Subjects were split into quartiles for level of copper loading as follows: <50 μg/m^2, 50-130 μg/m^2, 130-290 μg/m^2, and ≥290 μg/m^2.	**Outcomes:** Odds of child ALL did not significantly differ between copper loading quartiles in unadjusted or adjusted models. **Limitations:** Exposure measurement occurred, on average, 1.34 years after ALL diagnosis (or reference date for controls), so temporality cannot be inferred. The study did not account for other potential sources of metal

Table 2-6. Copper Exposure from Environmental Media and Health Outcome Associations in Human Studies

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, Covariates Considered and Adjustments:	Outcomes and Limitations
(CCL) Study (142 cases and 187 controls).	**Inclusion/Exclusion Criteria:** Subjects were enrolled in the CCL Study between Dec 1999-Nov 2007, ages 0-7 years old, and occupying the same home they occupied at the time of diagnosis (or similar reference date for controls). **Covariates Considered/Other Regression Adjustments:** Age at diagnosis (or reference date for controls), sex, race/ethnicity, annual household income, season of dust sampling, year of dust sampling (2001-2003 or 2004-2006).	exposure (inhalation, breastfeeding, diet, etc.). Biological intake of metals was not measured. Dust measurements could not account for variability in dust accumulation in the homes.

AD = Alzheimer's Disease, ALL = acute lymphoblastic leukemia, AS = ankylosing spondylitis, CCL = California Childhood Leukemia, CI = confidence interval, ESR = erythrocyte sedimentation rate, IRR = incidence rate ratio, MND = motor neuron disease, RA = rheumatoid arthritis, RR = relative risk, SMR = standardized mortality ratio, WBC = white blood count

Table 2-7. Serum Copper Levels and Health Outcome Associations in Human Studies

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, Covariates Considered and Adjustments:	Outcomes and Limitations
Cardiovascular		
Ford 2000 **Study Type:** Prospective cohort study of 4,574 adults aged 30-75 who participated in NHANES II from 1976-1980. Mortality data from coronary heart disease reported in NHANES (1976-1992) reviewed.	**Exposure:** Measured **c**opper concentration in blood serum. **Inclusion/Exclusion Criteria:** Subjects were NHANES participants from 1976-1992. Subjects who had prevalent heart disease, no serum copper measurement, missing covariate data, or were lost to follow up were excluded. **Covariates Considered/Other Regression Adjustments:** Age, sex, race, education level, smoking status, systolic blood pressure, serum cholesterol, serum high density lipoprotein cholesterol, body mass index, recreational activity, non-recreational activity, history of diabetes, white blood cell count.	**Outcomes:** 151/4,574 subjects died from coronary heart disease (CHD). Age-adjusted serum copper was 5% higher in subjects who died from CHD than in those who did not (129.8 µg/dl ± 3.7 SD versus 122.9 µg/dl ± 0.5 SD, p=0.072). Odds of death by CHD increased by 6% per 1 µmol/liter increase in serum copper in all models (multiple adjusted OR = 1.10, 95% CI: 1.05, 1.14). Hazard ratios for death by CHD and serum copper quartile showed that subjects in quartiles 3 and 4, but not 2, had significantly higher risk of death by CHD compared to quartile 1 (Q2 OR = 1.84, 95% CI: 0.93, 3.66; Q3 OR = 2.14, 95% CI: 1.21, 3.77; Q4 OR = 2.87, 95% CI: 1.57, 5.25). **Limitations:** Because deaths were identified by matching National Death Index information to Social Security files, some records may have been incorrectly matched or failed to match. Death certificates may be inaccurate. Participants were assumed to be alive if they could not be identified as being deceased.
Reproductive		
De Craemer et al. 2017 **Study Type:** 3 cross-sectional studies of Flemish adolescents (FLEHS I, II, and III), 2002-2015 (n = 1,659 for each study). Participant blood serum, urine, and hair sampled by study researchers.	**Exposure:** Copper levels in blood. Geometric mean blood copper was 699 µ/l (95% CI: 692, 706) for FLEHS I, 821 µ/l (95% CI: 810, 831) for FLEHS II, and 888 µ/l (95% CI: 873, 903) for FLEHS III. **Inclusion/Exclusion Criteria:** Subjects were adolescents aged 14-15. **Covariates Considered/Other Regression Adjustments:** Age, body mass index,	**Outcomes:** Blood copper was negatively associated with production of sex hormones, including E2, fE2, T, fl, and LH, and positively associated with SHBG in both FLEHS I and II cohorts (p<0.05 in all cases). In FLEHS I, blood copper was negatively associated with odds of male genital development (OR = 0.82, 95% CI: 0.692, 0.967). In FLEHS II, blood copper was negatively associated with the age of menarche (OR = -0.264, 95% CI: -0.387, -0.142). In FLEHS III, blood copper was negatively associated with odds of male pubic hair (OR =

Table 2-7. Serum Copper Levels and Health Outcome Associations in Human Studies

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, Covariates Considered and Adjustments:	Outcomes and Limitations
	contraceptive pill usage (females only), smoking status, hour of blood collection, fasting, passive smoking at home, urbanization, season, illness in the last 14 days, weekly alcohol consumption.	0.376, 95% CI: 0.23, 0.591) and male genital development (OR = 0.411, 95% CI: 0.251, 0.649). **Limitations:** The cross-sectional study design only accounts for very recent copper exposure and prevents inference of temporality between blood copper and sexual maturation.
Kasperczyk et al. 2016 Cross-sectional study of sperm quality in 65 fertile men in the southern region of Portland. Semen samples collected by study researchers.	**Exposure:** Copper concentration in seminal plasma (CuS). Participants were grouped into 2 exposure categories: low exposure (Cu-L = 10.2-21.7 μ/dl) and high exposure (Cu-H = 21.8-228 μ/dl). **Inclusion/Exclusion Criteria:** Subjects were males who were healthy, non-smoking, free of drug consumption (including antioxidant medications). **Covariates Considered/Other Regression Adjustments:** Age.	**Outcomes:** TOS Total oxidant status (TOS) was 243% higher, Mn-superoxide dismutase (SOD) activity was 125% higher, and median IL-10 level was 144% higher in the Cu-H group compared to the Cu-L group. Copper exposure was positively correlated with TOS and IL-10 and negatively correlated with granulocyte colony stimulating factor (G-CSF) and granulocyte-macrophage CSF (GM-CSF). Copper exposure was not significantly associated with sperm count, volume, motility, or morphology. **Limitations:** Cross-sectional study design only accounts for very recent copper exposure and prevents inference of temporality between copper exposure and sperm parameters. The sample size was small.
Developmental		
Yang et al. 2020 **Study Type:** Cross-sectional study of 734 mother-infant pairs in Wuhan, China, 2014-2015. Hospitals medical records of participants examined for birth outcomes.	**Exposure:** Serum copper concentrations in umbilical cord blood collected at birth/ **Inclusion/Exclusion Criteria:** Subjects were pregnant women who were residents in Wuhan city with a single gestation who were willing to take complete questionnaires and provide umbilical cord blood samples at delivery.	**Outcomes:** As a continuous variable, log-transformed copper in cord serum was linked with decreased birthweight z-score in all infants (β = -0.49, 95% CI: 0.69, -0.29) and term birth infants (β = -0.47, 95% CI: -0.67, -0.27). In quantile regression analysis, the association between increased copper concentration in cord serum and decreased birth weight was stronger below the 50th percentile for birthweight z-score.

Table 2-7. Serum Copper Levels and Health Outcome Associations in Human Studies

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, Covariates Considered and Adjustments:	Outcomes and Limitations
	Covariates Considered/Other Regression Adjustments: Maternal age, annual household income, pre-pregnancy BMI, parity, passive smoking during pregnancy, maternal weight gain during pregnancy, fetal sex, gestational age.	**Limitations:** The cross-sectional study design prevents the conclusion of a causal relationship between copper exposure in utero and birthweight. Dietary information was not collected from mothers, so various forms of arsenic (known to have varying effects on birthweight) in cord blood could not be distinguished and so could not be adjusted for.

ARF = acute renal failure, BMI = body mass index, CI = confidence interval, CHD = coronary heart disease; CVD = cardiovascular disease, EAF = electric arc furnace, FEF = mid-expiratory flows, FVC = forced vital capacity, IDR = interdecile range, IHD = ischemic heart disease, IMD = index of multiple deprivation, MND = motor neuron disease, PM = particulate matter, RR = relative risk, SMR = standardized mortality ratio, UF = ultrafine

2.2 DEATH

Inhalation

No toxicity studies were located regarding death of humans following inhalation exposure to copper. Copper in $PM_{2.5}$ has been associated with increased risk of cardiovascular disease mortality (Badaloni et al. 2017; Lavigne et al. 2019; Wang et al. 2020; Ostro et al. 2008; Valdes et al. 2012) and ischemic heart disease mortality (Badaloni et al. 2017; Lavigne et al. 2019, Ostro et al. 2015; Valdes et al. 2012), and cerebrovascular disease mortality (Valdes et al. 2012). Ostro et al. (2008, 2015) specifically noted an increased risk of mortality from cardiovascular disease among Hispanics, and mortality from ischemic heart disease among a cohort of teachers in California (Ostro et al. 2015); however, it is unclear whether it is the copper that is causing these effects, rather than $PM_{2.5}$, which is independently associated with adverse cardiovascular health outcomes, including cardiovascular mortality (EPA 2019). Increased risk of coronary heart disease death was associated with elevated serum copper levels among adult participants from NHANES (Ford 2000). Further study-specific information may be found in Table 2-4; it should be noted that the quality of these studies differs, and study limitations are included.

Acute inhalation LC_{50} values were 45 and 109 mg Cu/m^3 for female and male rats, respectively, following a 4-hour exposure to a copper-containing herbicide (Rush 1991). No death was reported in rats exposed for 4 hours to 1,662 mg Cu/m^3 as copper oxide aerosols (Holbert 1990). In mice exposed to copper sulfate with a Streptococcus aerosols challenge, mortality ranged from 54–70% higher compared to controls, and the mean survival time decreased by 4.2–5.9 days following a single 3-hour exposure to 0.56 mg/m^3 (Drummond et al. 1986). Mortality was one of several indicators that showed impaired immune response to the challenge resulting from copper sulfate exposure (see Section 2.14). When exposed to 0.13 mg/m^3 for 3 hours/day, 5 days/week for 2 weeks, mortality was 25-31% higher and survival time was 1.3–1.5 days lower, compared to controls (Drummond et al. 1986).

Oral

Several case studies have reported death following ingestion of large doses of copper sulfate (Sharma 2011; Gupta et al. 2018; Griswold et al. 2017; Chuttani et al. 1965). 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 (Gupta et al. 2018; Griswold et al. 2017). 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. Study authors reported a significant correlation between whole copper levels and the severity of manifestations among the 48 individuals (Chuttani et al. 1965). Deaths, likely due to central nervous system depression and hepatic or renal failure, have also been reported in individuals ingesting "spiritual green water," which contains ≥100 mg Cu sulfate/L (Akintonwa et al. 1989). A prospective cohort study found that the odds of death from cardiovascular disease increased by 6% per 1 µmol/L increase in serum copper (Ford 2000). The study used serum data collected from individuals who participated in a medical examination for NHANES II (1976-1980) and were followed though 1992 (NHANES III). Vital status was determined by matching participant information to Social Security Administration data and the National Death index. The NHANES serum copper levels most likely reflect a combination of dietary intake and occupational exposures (Ford 2000).

An oral LD_{50} for mice was reported as 39.8 mg Cu/kg, however, only 2 mice were tested (Kadammattil et al. 2018). Oral LD_{50} values of 42 and 37 mg Cu/kg were reported in male and female rats, respectively, exposed to an herbicide with 8% elemental copper (Rush 1990b). Following exposure to an algaecide containing 8% elemental copper, oral LD_{50} values of 94 and 118 mg Cu/kg were reported for male and female rats, respectively (Rush 1990c). Increased mortality was observed in rats fed a diet containing 4 mg/g of added copper (133 mg Cu/kg/day) for 1 week, compared to controls (Boyden et al. 1938). Reduced food intake, possibly the result of taste aversion, contributed to the deaths. No death was reported in 10 rats from a single oral dose of 4,034 mg Cu/kg/day as copper oxide (Kuhn 1989a).

Oral exposure animal studies examining copper lethality reported mix results following intermediate- or chronic-durations. Among 10 rats orally exposed to 31 mg Cu/kg/day in water for 15 days, 100% mortality was reported before the end of study period (NTP 1993). However, no death was reported in rats when exposed for 15 days in feed to doses up to 325 mg Cu/kg/day. This difference of vehicle is further demonstrated in the 13-week study where no death was reported in male and female rats exposed daily to 140 and 134 mg Cu/kg/day in feed, respectively (NTP 1993). Similar results were seen in mice. One of five male mice died following exposure to 58 mg Cu/kg/day in water for 15 days (NTP 1993). When exposed to copper in feed, no death was reported in male and female mice exposed daily to 717 to 781 mg/kg/day for 15 days or to 815 to 1058 mg Cu/kg/day for 13 weeks (NTP 1993). 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). Following exposure of 5 New Zealand white rabbits to 30 mg Cu/kg/day as copper hydroxide via gavage for 23 days, 2 were found dead. In the same report, 2 of 8 pregnant rabbits died following exposures on gestation days 7 to 28 (Munley 2003a). In a similar study, 3 of 22

pregnant New Zealand white rabbits were reported dead when exposed to 18 mg Cu/kg/day via gavage over gestation days 7 to 28 (Munley 2003b). No deaths were reported at 15 mg Cu/kg/day (Munley 2003a) or at 9 mg Cu/kg/day (Munley 2003b).

Dermal

No studies were found regarding death in humans following dermal exposure to copper.

One New Zealand white rabbit was reported dead after a 24-hour dermal exposure to 1,613 mg Cu/kg as copper oxide applied to its shaved dorsal epithelial (back skin) surface (Kuhn 1989b). The study author concluded the acute dermal LD_{50} to be >1,613 mg Cu/kg. No death was reported in New Zealand white rabbits exposed to an herbicide (8% elemental copper) with a dose of 160 mg Cu/kg (Rush 1990a). No death was reported in rats dermally exposed to ≤181 mg Cu/kg, as copper 8-quinolinolate, for 6 hours/day, 5 days/week for 4 weeks, applied to the shaved back skin surface (Hagemann 1992).

Other Routes

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).

2.3 BODY WEIGHT

Inhalation

No studies were located regarding body weight effects in humans or animals following inhalation exposure to copper.

Oral

Authors did not observe any effects on body weight in a drinking water study in human females exposed to a daily dose of up to 0.1 mg Cu/kg/day following a 2-week exposure period (Pizarro et al. 1999). In addition, no changes in body weight were reported in infants following a daily dose of up to 0.319 mg Cu/kg/day in drinking water for 9 months (Olivares et al. 1998).

No body weight effects were reported in rats following exposure to 50.9 mg Cu/kg/day for 30 days

(Khushboo et al. 2018). Intermediate-duration dietary exposure studies reported 12–27% decreases in body weight gain and 10-28 % decreases of body weight in rats following exposure to 25.5–140 mg Cu/kg/day for 15–91 days (Kumar et al. 2015, 2016a, 2016b; NTP 1993; Rana and Kumar 1980; Tian et al. 2019); in mice following exposure to 10.7–398 mg Cu/kg/day for 15–133 days (Kvietkauskaite et al. 2004; NTP 1993); and in pigs following exposure to 16.5–18.7 mg Cu/kg/day for 46–49 days (Suttle and Mills 1966). An unspecified decrease in body weight gain was observed in pigs exposed to 2.3 mg Cu/kg/day for 88 days (Kline et al. 1971). No effects on body weight were reported in rats at doses of 8–325 mg Cu/kg/day (Abe et al. 2008; Epstein et al. 1982; Kalita et al. 2020; Kumar et al. 2019; NTP 1993; Seven et al. 2018); in mice at doses of 2.4–781 mg Cu/kg/day (Cheng et al. 2020; Kvietkauskaite et al. 2004; NTP 1993); in pigs at 1.7 mg Cu/kg/day (Kline et al. 1971); in rabbits at 6-30 mg Cu/kg/day (Munley 2003a, 2003b; Shen et al. 2005); and in guinea pigs at 9.6 mg Cu/kg/day (Seffner et al. 1997). Additionally, no exposure-related changes in maternal body weight were seen in rats exposed to 130 mg Cu/kg/day for up to 73 days, prior to mating and during gestation (Haddad et al. 1991). Normally an increase in maternal body weight is expected during gestation. A chronic study found no biologically significant body weight effect in mice exposed to 42 mg Cu/kg/day as copper gluconate in drinking water (Massie and Aiello 1984). A 2-year chronic study in monkeys also found no effects on body weight following exposure to 0.77–1.05 mg Cu/kg/day (Araya et al. 2012).

Dermal

No studies were found regarding body weight effects in humans following dermal exposure to copper.

One dermal toxicity study in albino rats did not report any significant differences in body weight or food intake between groups following dermal exposure to 0, 9, 36, or 181 mg Cu/day for 6 hours/day, 5 days/week for 4 weeks (Hagemann 1992). The substance, copper 8-quinolinolate was applied as an ointment to the shaved back skin surface.

2.4 RESPIRATORY

Inhalation

In humans, copper is a respiratory irritant. Workers exposed to copper dust have reported symptoms such as coughing, sneezing, thoracic pain, and a 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). 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 to introduction of mechanization and added protective measures for workers. By the third year, the levels were estimated to average 111 mg Cu/m^3. For reference, the permissible exposure limit (PEL) by OSHA for an 8-hour time weight average (TWA) exposure to copper dusts and mists in general industry is 1 mg/m^3 (OSHA 2020a).

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. Two case studies reported non-occupational respiratory effects following copper inhalation in humans. A 2-year-old female child developed an acute respiratory distress syndrome with dyspnea, bilateral hyperinflation, and interstitial infiltrates of the lungs following inhalation of copper dust (Donoso et al. 2007). These effects were further indicated by cyanosis on the patient. A 24-year-old man developed a deviated septum with persistent sinus pressure and rhinorrhea after spilling molten copper on his face shield and inhaling the associated fumes (Gibson et al. 2011). Multiple studies examined the associations between copper levels in particulate matter and associated respiratory outcomes among general populations. A decline of copper in particulate matter was associated with improved forced vital capacity in a prospective cohort study in the Netherlands (Boogaard et al. 2013). Increased risk of allergic sensitization was positively associated in children exposed to the copper in airborne PM_{10} aerosols in either their current home address or home address at birth (Gehring et al. 2015). Increased odds of asthma symptoms were positively associated with the estimated copper concentrations in PM_{10} at current home address. Measures of forced expiratory volume in one second was negatively associated with copper concentrations in PM_{10} at the child's current home address and with copper concentrations in $PM_{2.5}$ at the birth home address (Gehring et al. 2015). These epidemiological studies examining the occurrence of adverse respiratory health effects from inhalation copper exposure are summarized in Table 2-4. For this table, note that the study quality varies; study limitations are included.

Copper is considered the etiologic agent in an occupational disorder referred to as "vineyard sprayer's lung." This condition is found in vineyard workers that used an anti-mildew agent known as the "Bordeaux mixture" that contains 1– 2.5% copper sulfate and the pH neutralized with 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). Through alveolar lavage and biopsy, case reports observed 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 data 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, as compared to rural workers from the same geographic region who did not work in the vineyards (Plamenac et al. 1985). The data are limited and there is no accompanying concentration-response information to correlate with the histological data.

The potential for copper to induce respiratory effects has been evaluated in mice, hamsters, and rabbits. Decreased cilia beating was observed in Syrian-Golden hamsters exposed to 3.3 mg Cu/m^3 as copper sulfate for 3 hours (Drummond et al. 1986). This effect was not observed in similarly exposed CD-1 mice. However, in mice repeatedly exposed to 0.12 mg Cu/m^3 as copper sulfate for 3 hours/day, 5 days/week for 1–2 weeks, mice showed increased alveolar wall thickening (Drummond et al. 1986). The severity of the effect increased with the duration of exposure. This alveolar wall thickening was not observed in similarly exposed hamsters (Drummond et al. 1986).

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 or morphological alterations were observed in the alveolar macrophages of similarly exposed rabbits (Johansson et al. 1983). Combined, the mice, hamster, and rabbit studies indicate that there may be species differences in response to the inhalation of particulate copper. However, such a difference could also be influenced by the study (i.e., particle size and composition).

Oral

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 being tachypnea (fast breathing) and dyspnea (labored breathing) (Sood and Verma 2011; Gupta et al. 2018; Franchitto et al. 2008; Higny et al. 2014; Sinkovic et al. 2008; Hassan et al. 2010; Cho et al. 2018; Gunay et al. 2006; 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 (Gamakaranage et al. 2011; Franchitto et al. 2008). 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).

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 to 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. In pregnant rabbits, post-mortem evaluation of the lungs revealed no exposure-related changes following daily exposure to 7.5–30 mg Cu/kg/day, as copper hydroxide, from gestation days 7 to 28 (Munley 2003a). In a similar study by Munley (2003b), pregnant rabbits were given 18 mg Cu/kg/day as copper hydroxide via gavage. At necropsy, brown liquid was present in the chest cavity of 3 rabbits that died before the exposure period ended, including the one that experienced irregular respiration prior to death. Dark discoloration and/or mottling of lung tissue was observed among the three rabbits (Munley 2003b).

Dermal

Two case studies examined respiratory effects in humans following dermal exposure to copper. A 2-year-old female spilled a copper powder resulting in contact with her facial skin and some inhalation of the powder (Donoso et al. 2007). Her development of acute respiratory distress syndrome was attributed to inhalation of the powder. No effects were attributed to the dermal exposure. A 24-year-old man spilled molten copper on his face at work after his face shield was blown off. He developed a deviated septum with persistent sinus pressure and rhinorrhea (Gibson et al. 2011). This effect was likely caused by inhalation of the substance, but dermal exposure could not be conclusively ruled out as a contributing factor to the deviated septum and rhinorrhea.

No evidence of exposure-related gross lesions was observed in the lungs or trachea of rats dermally exposed to 0, 9, 36, or 181 mg Cu/kg, as copper 8-quinolinolate applied to the shaved skin on their backs for 6 hours/day, 5 days/week for 4 weeks (Hagemann 1992).

Other Routes

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)

2.5 CARDIOVASCULAR

Inhalation

Human data on cardiovascular effects from inhalation exposure are limited to a few epidemiological studies. 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/m^3) to the outcomes of workers in 1972 when air concentrations were lower (≤111 mg/m^3). 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). There are a few epidemiologic studies that have looked at various cardiovascular-related mortality outcomes (Badaloni et al, 2017; Occelli et al. 2020; Ostro et al. 2008, 2015; Lavigne et al. 2019; Wang et al. 2020; Valdes et al. 2012). These studies have reported associations between copper air pollution, and cardiovascular mortality and heart disease. The details from these studies are presented in Section 2.1, Table 2-4.

No dose-response toxicity studies were located regarding cardiovascular effects in animals following inhalation exposure to copper.

Oral

Increased risk of death from coronary heart disease was associated with elevated serum copper levels among adult participants from NHANES (Ford 2000); for more information see Table 2-7. A number of case studies have 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 (Sood and Verma 2011; Gupta et al. 2018; Franchitto et al. 2008; Sinkovic et al. 2008; Higny et al. 2014; Cho et al. 2018; Gunay et al. 2006; Griswold et al. 2017). Conversely, two case studies of patients who initially presented with blue-colored vomitus 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 (Higny et al. 2014; Hassan et al. 2010). 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).

Male Wistar rats exposed to 50.9 mg Cu/kg/day of copper sulfate for 30 days showed flabby, enlarged, congested hearts upon gross pathology (Khushboo et al. 2018). In another study, male Wistar rats

exposed to the same dose of 50.9 mg Cu/kg/day of copper sulfate pentahydrate for 90 days had a systolic blood pressure 33% higher than controls by the end of the exposure period (Arafa et al. 2019). No histological alterations were observed in the hearts of rats exposed to 29 to 325 mg Cu/kg/day for 15 to 90 days, or in mice exposed to 24 to 1,058 mg Cu/kg/day for 15 to 90 days (NTP 1993).

Dermal

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

No evidence of exposure-related gross lesions was observed in the heart or aorta of rats dermally exposed to 0, 9, 36, or 181 mg Cu/kg, as copper 8-quinolinolate, on shaved back skin for 6 hours/day, 5 days/week for 4 weeks (Hagemann 1992). The hearts were weighed and no exposure-related differences in relative heart weight were noted for exposed and non-exposed rats.

Other Routes

A 40-year-old woman developed toxic myocarditis followed by a 2-minute-long cardiac arrest after intentionally rectally inserting an unknown amount of copper sulfate (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).

2.6 GASTROINTESTINAL

Based on a systematic evaluation of the literature, gastrointestinal toxicity is presumed to be a consistent health effect of oral exposure to dissolved copper salts. A conclusion on gastrointestinal toxicity could not be determined because of the lack of data in human or animals following inhalation or dermal exposure to copper. The full results of the systematic review for the gastrointestinal endpoint are presented in Appendix C.

Inhalation

In workers involved in grinding and sieving copper dust, anorexia, nausea, and occasional diarrhea were reported (Suciu et al. 1981); exposure levels ranged from 111 to 464 mg Cu/m^3 over a 3-year period.

While initial exposure was via the inhalation route, it is possible that the observed gastrointestinal effects were due to oral exposure to copper. Ingestion probably 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).

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

Oral

There are numerous reports of acute gastrointestinal effects in humans after intentional or accidental ingestion of copper substances. The most common effects include abdominal pain, nausea, vomiting, diarrhea, and melena (black stool), which typically occur shortly after ingestion and are not persistent (Araya et al. 2001, 2003a, 2003b, 2003c; Gotteland et al. 2001; Knobeloch et al. 1994, 1998; Olivares et al. 2001; Pizarro et al. 1999, 2001). Gastrointestinal ulcerations and hemorrhaging were also observed following copper sulfate ingestion in several case studies (Gamakaranage et al. 2011; Du and Mou 2019; Franchitto et al. 2008; Griswold et al. 2017; Malik and Mansur 2011; Lubica et al. 2017). There have been several reports of upper gastrointestinal effects, including oral mucositis, pharyngeal edema, and odynophagia, following copper sulfate ingestion (Higny et al. 2014; Hassan et al. 2010). 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).

Several epidemiological studies examined the occurrence of gastrointestinal symptoms in communities exposed to elevated levels of copper in drinking water; they are described in detail in Table 2-5. Gastrointestinal symptoms, including abdominal pain and diarrhea, were associated with copper exposure in several populations (Eife et al. 1999; Knobeloch et al. 1994, 1998; Pizarro et al. 2007). Conversely, two studies did not find significant associations of gastrointestinal symptoms with copper in drinking water (Buchanan et al. 1999; Pettersson et al. 2003). Specifically, Buchanan et al. (1999) observed no increased risk of gastrointestinal symptoms when comparing household waters with copper concentrations <1.3, 2 to 3, or >3 mg Cu/L (Buchanan et al. 1999). Pettersson et al. (2003) observed no increased hazard of diarrhea or vomiting among homes with children and copper concentrations in water ranging from 0.01 to 5 mg Cu/L.

Repeated exposure studies conducted in adults exposed to copper in drinking water have observed gastrointestinal symptoms (Araya et al. 2003b, 2004; Olivares et al. 2001; Pizarro et al. 1999, 2001). The doses calculated from these studies represent the exposure from copper in drinking water only. Several studies did survey participants on their diets; however, copper intake from normal diets was not considered in the dose. These studies are presented in Table 2-2 and Figure 2-3.

Symptoms of gastrointestinal upset following acute exposure to copper are suspected to be a direct contact effect, in that the symptoms result from the maximum serum concentration (C_{max}) of copper in the gastrointestinal system at a time point rather than the 24-hour intake (Donohue 1997). A study by Wang and Borison (1951) hypothesized a biphasic mechanism of copper sulfate-induced emesis. The effect of copper sulfate on the peripheral nervous system followed by a systemic effect on the central nervous system was associated with the absorbed copper intake (Horn et al. 2014; Wang and Borison 1951). Several studies in mammals have demonstrated that copper sulfate-induced emesis results from contact in the stomach mediated by the vagus nerve (Makale and King 1992) and shown that 5-HT_4 receptors and abdominal vagal afferents are closely associated and play a role in inducing vomiting (Bhandari and Andrews 1991; Fukui et al. 1994).

A study by Pizarro et al. (1999) demonstrated a dose-response relationship between copper sulfate and gastrointestinal symptoms (nausea, vomiting, and abdominal pain) in healthy adult women. Each study participant consumed either 0, 1, 3, or 5 mg/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; dosing for each exposure group was calculated as 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 copper-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). This study and its limitations are further described in Table 2-5. Abdominal pain, nausea, and/or vomiting have also been 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 copper sulfate or copper oxide, with the same amount of copper in each (Pizarro et al. 2001). Both Pizarro et al. (1999, 2001) studies reported no associations between copper levels in drinking water and the incidence of diarrhea. In Pizarro et al. (1999), 8 of 12 cases of diarrhea presented within the first 2 weeks of exposure in all exposure groups, and then the number of cases declined, regardless of the copper

concentration. In Pizarro et al. (2001), 7 of 10 cases of diarrhea occurred during the first half of the exposure period, differing from other gastrointestinal effects, like nausea, which were uniformly distributed during the study period. Diarrhea appears to be an effect of copper exposure but does not appear to be concentration- or dose-dependent in these studies.

In a 2-month study by Araya et al. (2003b), 19.7% of male and female adults exposed to 0.138 mg Cu/kg/day (6 mg Cu/L) reported at least one gastrointestinal symptom, among nausea, vomiting, diarrhea, and abdominal pain, at some point during the exposure period. Incidence of symptoms was higher for this dose group compared to subjects exposed to ≤0.092 mg Cu/kg/day (2 or 4 mg Cu/L) (Araya et al. 2003b). Araya et al. (2004) observed that at the end of the first week of exposure, gastrointestinal symptoms (nausea, abdominal pain, vomiting, and diarrhea) were significant for females exposed to ≥0.106 mg Cu/kg/day (4 mg Cu/L) and males exposed to ≥0.169 mg Cu/kg/day (6 mg Cu/L). The study 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; however, results did not distinguish between gastrointestinal symptoms. In Araya et al. (2004) nausea and vomiting were the only symptoms that appeared to show a clear dose-response relationship with copper concentration in drinking water. As the duration of exposure increased, the concentration in water necessary to achieve a positive gastrointestinal response increased.

Multiple single-exposure studies have observed that nausea is adults' most reported symptom following ingestion of copper in drinking water (Araya et al. 2001, 2003a, 2003c; Gotteland et al. 2001; Olivares et al. 2001). Olivares et al. (2001) 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. Two studies by Araya et al. (2001; 2003c) reported a threshold of 6 mg Cu/L for increased incidence of nausea. In a multinational 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, with a NOAEL of 0.06 mg Cu/kg for nausea. The 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 was confirmed in adults exposed to 10 mg Cu/L of drinking water, as 9/30 adults reported nausea following a single dose of 0.046 mg Cu/kg (Araya et al. 2003a), and 6/31 adults reported nausea following a dose of 0.03 mg Cu/kg (Gotteland et al. 2001). Olivares et al. (2001) found that the threshold for nausea increased to 8 mg Cu/L when copper was given in an orange-flavored juice (instead of water). Increased incidence of vomiting

was observed in adults following exposure to single doses of 0.018 mg Cu/kg (6 mg Cu/L) and 0.03 mg Cu/kg (10 mg Cu/L) (Olivares et al. 2001; Gotteland et al. 2001).

A study of fifty-six healthy babies who received 2 mg/L of copper sulfate in water daily for 9 months did not report 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 breast-fed babies had symptoms. Controls were exposed to copper doses ranging between 0.123 to 0.158 mg Cu/kg/day, and experimental infants to doses ranging from 0.248 to 0.319 mg Cu/kg/day (Olivares et al. 1998).

Two studies examined physiological alteration in the intestines of adults exposed to copper in drinking water (Araya et al. 2003a; Gotteland et al. 2001). Araya et al. (2003a) observed delayed gastric emptying time, as copper induced a significant delay in increasing the antral area of the stomach in adults exposed to a single dose of 0.046 mg Cu/kg (10 mg Cu/L of drinking water). The antral area is the lower part of the stomach that surrounds the portal entry to the small intestines. The effect occurred during the first hour after fasting adults (15 males, 15 females) were given a bolus of 10 mg Cu/L of copper sulfate solution. Three men and six women experienced nausea following ingestion. The delay in decreasing antral area indicated the continued presence of the ingested solution in the stomach and reflected an increase in the gastric emptying time for both males and females. There was no significant relationship between the delay in gastric emptying and the presence or absence of nausea (Araya et al. 2003a). A study by Gotteland et al. (2001) was designed to evaluate whether there was a change in the permeability of the gastric and intestinal mucosa following exposure to a bolus intake of a 10 mg/L copper sulfate solution. Twenty percent of subjects experienced nausea, and 5% reported vomiting. The copper sulfate solution contained either sucrose or lactulose/mannitol mixture to determine if copper sulfate influenced permeability of the stomach and intestinal membrane. There was a 36.5% increase in gastric permeability to sucrose following the bolus ingestion of 10 mg Cu/L copper solution, but a dose of 0.03 mg Cu/kg elicited no adverse effect. However, no alterations in intestinal permeability to lactulose/mannitol were found. The increased gastric permeability was independent of gastrointestinal nausea/vomiting response (Gotteland et al. 2001).

Gastrointestinal effects have been reported in multiple animal studies. Histological lesions and disruptions in intestinal microbiota homeostasis were reported in the cecum and rectum of mice exposed to 2.4 mg Cu/kg/day, as copper chloride, for 90 days (Cheng et al. 2020). The histological observations included increased thickness of outer muscularis and smooth muscle fiber, widened submucosa, decreased goblet cells, blunting of intestinal villi, and severe atrophy of the central lacteal. Changes in microbiota

homeostasis were reflected by changes in the microbial strain composition of the biota, with some strains increasing and others decreasing. The microbial changes were observed in the cecum and more pronounced in the rectum of the mice (Cheng et al. 2020). A different study showed focal intestinal ulceration in mice exposed to 4 mg Cu/kg/day for 7 days; however, no effects were seen at lower doses (Kadammattil et al. 2018). No gastrointestinal effects were seen in shrews exposed to a single dose of 4 mg Cu/kg; however, 15 episodes of emesis (vomiting) were reported in 4/4 shrews exposed to 48 mg Cu/kg by gavage (Yamamoto et al. 2004). In the same study, no clinical signs of gastrointestinal toxicity were reported in rats at 4–16 mg Cu/kg. At a high dose of 50.9 mg Cu/kg/day, signs of copper toxicity in rats included thickened stomach wall with corrugated mucosa (Khushboo et al. 2018).

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, 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 (NTP 1993). No effects were seen at lower doses of copper in the diet of rats and mice. Animals exposed to copper in drinking water did not show any gastrointestinal effects, including in rats exposed to doses up to 31–36 mg Cu/kg/day and in mice exposed to doses up to 24–36 mg Cu/kg/day, both for 15 days (NTP 1993). Diarrhea was seen in pregnant rabbits exposed to 6–30 mg Cu/kg/day as copper hydroxide administered via gavage from gestation days 7–28 (Munley 2003a, 2003b). One rabbit who died prior to the end of the exposure period showed stomach hemorrhage at necropsy (Munley 2003a). Three rabbits exposed to 18 mg Cu/kg/day that died prior to the end of the exposure exhibited stomach hemorrhage, ulceration, or both (Munley 2003b). With the 18 mg Cu/kg/day diet, diarrhea preceded death in one rabbit, and fetal abortion occurred in two rats; red, discolored stomach lining was seen in one of the rats who aborted (Munley 2003b).

Dermal

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

No evidence of exposure-related gross lesions was observed in the stomach, esophagus, pancreas, small intestine, or large intestine of rats dermally exposed to 0, 9, 36, or 181 mg Cu/kg, as copper-8-quinolinolate applied to shaved areas on their backs for 6 hours/day, 5 days/week for 4 weeks (Hagemann 1992).

2.5 HEMATOLOGICAL

Inhalation

Decreased hemoglobin and erythrocyte levels were observed in workers exposed to airborne copper dust levels of 0.64–1.05 mg/m^3 (Finelli et al. 1981). The OSHA PEL for an 8-hour exposure to copper dusts and mist in general industry is 1 mg/m^3 (OSHA 2020a). Results of hair analysis reveal that the workers had also been exposed to iron, lead, and cadmium (Finelli et al. 1981). A 2-year-old female child who accidentally inhaled a copper powder developed hypoxemia and hemolytic anemia (Donoso et al. 2007).

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

Oral

Numerous case studies have reported hematological effects in humans following ingestion of copper-containing substances. The most common effects are hemolytic anemia, hemoglobinemia, methemoglobinemia, leukocytosis, and reduced reticulocyte count (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; Lubica et al. 2017; Malik and Mansur 2011; Mortazavi and Jafari-Javid 2009; 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 (Du and Mou 2019; Hassan et al. 2010; Malik and Mansur 2011; Sinkovic et al. 2008; Yang et al. 2004). In a study where 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).

Several studies examined the hematological effects of copper in rats, mice, pigs, and rabbits following intermediate-duration exposures. In rats exposed for an intermediate duration (20-90 days) to doses of 25.5-39.8 mg Cu/kg/day, effects observed included decreased hemoglobin (Kumar et al. 2015); anemia evidenced by reductions in hemoglobin and red blood cell levels (Kumar and Sharma 1987); and decreased erythrocyte, hemoglobin, and hematocrit levels (Rana and Kumar 1980). In female rats exposed to 196 mg Cu/kg/day for 13 weeks, depletion of hematopoietic cells in bone marrow was observed, and in female rats exposed to 66 mg Cu/kg/day for 15 days, effects included decreases in hematocrit, hemoglobin, reticulocyte, and mean cell volume levels and an increase in platelet levels (NTP 1993). In male rats exposed to high doses of 325 mg Cu/kg/day for 15 days, and in female rats exposed to 134 mg

Cu/kg/day for 13 weeks, no hematological effects were observed (NTP 1993). No hematological effects were observed in mice exposed to 10.7 mg Cu/kg/day ad libitum for 19 weeks or 1058 mg Cu/kg/day for 13 weeks (Kvietkauskaite et al. 2004; NTP 1993). One study in rabbits observed changes in blood composition including unspecified decreases in neutrophils, eosinophils, platelets, monocytes, and basophils at 16 mg Cu/kg/day for 50 days (Shen et al. 2005). In pigs, decreased hemoglobin levels and increased erythrocyte count was seen with 16.5–18.7 mg Cu/kg/day after 46-49 days of exposure (Suttle and Mills 1966). Kline et al. (1971) identified a NOAEL of 2.7 mg Cu/kg/day for hemoglobin levels in pigs following 88 days of exposure. Chronic-duration exposure studies in monkeys found no hematological effects after exposure to a daily dose of 0.77 mg Cu/kg/day for 3 years; however, lower hemoglobin levels were observed with a dose of 1.05 mg Cu/kg/day when compared to the controls (Araya et al. 2012).

Dermal

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).

No evidence was observed of exposure-related differences in hematological parameters in rats exposed to 0, 9, 36, or 181 mg Cu/kg, as copper 8-quinolinolate, on shaved areas of their backs for 6 hours/day, 5 days/week for 4 weeks (Hagemann 1992). Hematological parameters measured included hemoglobin and hematocrit levels.

Other Routes

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 3 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 (Motlhatlhedi et al. 2014).

2.8 MUSCULOSKELETAL

Inhalation

No studies were located regarding musculoskeletal effects in humans or animals following inhalation exposure to copper.

Oral

Data on musculoskeletal effects in humans following oral exposure to copper is limited but was observed in several case studies. Rhabdomyolysis (breakdown of skeletal muscle) was reported in two cases, 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).

Impaired muscle strength was observed in rats exposed to 4 mg Cu/kg/day for 16 weeks, measured by the rotarod test (Kumar et al. 2019). Depressed skeletal growth has been observed in rats administered 39.8 mg Cu/kg/day via gavage; tail length was used to assess skeletal growth (Rana and Kumar 1980).

Dermal

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

No evidence of exposure-related gross lesions was observed in the skeletal muscle, femur joint, and sternum of rats dermally exposed to 0, 9, 36, or 181 mg Cu/kg, as copper 8-quinolinolate, on shaved back skin for 6 hours/day, 5 days/week for 4 weeks (Hagemann 1992).

2.9 HEPATIC

Based on a systematic evaluation of the literature, hepatic toxicity is a suspected health effect of exposure to copper. The full results of the systematic review for the hepatic endpoint are presented in Appendix C.

Inhalation

Hepatomegaly was observed in workers involved in grinding and sieving copper dust (Suciu et al. 1981); the exposure levels ranged from 111 to 464 mg Cu/m^3 over a 3-year period during which exposure decreased over time. One case study reported elevated aspartate aminotransferase (AST) and bilirubin in a

2-year-old female child who accidentally inhaled a copper powder and got some on her face (Donoso et al. 2007).

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

Oral

Numerous case reports are available on the hepatic effects in humans following accidental or intentional ingestion of copper substances, including copper sulfate, copper oxychloride, and copper-8-hydroxyquinolate. The most common effect was altered liver enzyme activity, including AST, alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (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; Yadla et al. 2015; Yang et al. 2004). Without providing many details, liver impairment was reported in two cases: 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, plus 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 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/L (Eife et al. 1999). In a study of seven adults receiving capsules containing 0.15 mg Cu/kg/day as copper gluconate, no significant alterations in serum AST, ALP, serum gamma glutamyl transferase (GGT), or LDH activities were found (Pratt et al. 1985).

Several studies examined liver function in infants exposed to elevated levels of copper in drinking water. A NOAEL for liver effects was identified in a study of infants (3 months of age at study initiation) exposed to 0.3 mg Cu/kg/day as copper sulfate in drinking water for 9 months (Olivares et al. 1998). No alterations in total bilirubin levels or serum ALT, AST, or GGT activities were found. A higher

percentage of copper-exposed infants (30.4%) were dropped from the study, as compared to the control group (11.1%). The reasons for being withdrawn from the study were blood sampling refusal (8 infants in the copper group and 2 infants in the control group), protocol transgression (4 infants in the copper group and no infants in the control group), and change of address (5 infants in the copper group and 1 infant in the control group). 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 or alterations in test results from liver ultrasound imaging (see Table 2-5) (Zietz et al. 2003a, 2003b).

A few studies examining hepatic effects in adults exposed to copper did not find significant changes resulting from copper exposure. Liver enzyme levels (ALT, GGT) were not significantly different from baseline levels in female adults following exposure to drinking water containing 0 to 5 mg Cu/L equivalent to doses of 0.0006 to 0.1 mg Cu/kg/day for 2 weeks (Pizarro et al. 1999) or exposure to 5 mg Cu/L equivalent to 0.1 mg Cu//kg/day for 1 week (Pizarro et al. 2001).. Similarly, no alterations in liver function were seen in adults exposed to doses of 0.046 to 0.138 mg Cu/kg/day in drinking water for 2 months (Araya et al. 2003b). No alterations in liver function, evidenced by no change in liver enzyme measurements of alanine aminotransferase and L-GGT, were seen in 11 males and 11 females whose diets were supplemented with 3 mg Cu/day for 6 weeks (O'Connor et al. 2003). In this study, participants served as their own controls, and normal dietary copper intake during the study period was 0.018 mg Cu/kg/day for males and 0.017 mg Cu/kg/day for females. Summing the normal dietary and supplemental exposure resulted in a dose of 0.058 mg Cu/kg/day and 0.067 mg Cu/kg/day for males and females, respectively (O'Connor et al. 2003).

Wilson's disease, Indian childhood cirrhosis (ICC), and idiopathic copper toxicosis (ICT) are diseases largely defined by accumulation of copper in the liver. While Wilson's disease is considered to be a genetic disorder, the etiologies of ICC and ICT are less clear.

Wilson's disease. Wilson's disease is a rare, autosomal, recessive genetic disorder with a prevalence of approximately 30 to 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 copperiedus (which refers to copper deposits in the cornea known as Kayser-Fleischer rings) (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 (Taylor et al. 2020; Scheinberg and Sternlieb 1996).

Indian childhood cirrhosis. 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; Taylor et al. 2020). 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 et al. 1983; Tanner 1998). 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; Taylor et al. 2020). 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).

Idiopathic copper toxicosis. While no precise mechanism is identified, 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, 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.

The hepatotoxicity of copper in animals is described and investigated in numerous acute- and intermediate-duration oral exposure studies. The majority of these studies used rats; a small number of studies used pigs, mice, rabbits, guinea pigs, and monkeys. Additionally, a number of studies examined animals with genetic defects similar to Wilson's disease, primarily in Long-Evans Cinnamon (LEC) rats and Bedlington terrier dogs. Studies examining animals with these genetic defects were not included in our database on copper toxicity for healthy humans. Additionally, it would be inappropriate to consider the results of these studies for MRL derivation, therefore they are not discussed in this chapter.

Seven-day oral exposure studies in rats and mice identified hepatic effects following exposure to varying doses of copper sulfate (Alhusaini et al. 2018a, 2018b; Kadammattil et al. 2018). Histological observation of the liver in rats showed unspecified reduced relative liver weights, and massive cellular degeneration and necrosis of hepatocytes after oral doses of 39.8 mg Cu/kg/day (Alhusaini et al. 2018b). These findings were supported by elevated serum biomarkers of ALT, AST, LDH, C-reactive protein (marker of inflammation), hepatic nitric oxide (NO), lipid peroxidation, protein expression of cyclooxygenase 2 (COX-2), and DNA fragmentation. Additionally, glutathione (GSH) and superoxide dismutase (SOD) levels were decreased (Alhusaini et al. 2018b). Findings for ALT, NO, malondialdehyde (MDA), GSH, and SOD indicating liver damage were validated at a dose of 119 mg Cu/kg/day and associated with elevated caspase-3, reduced interleukin-10, and hepatic CYP450 (Alhusaini et al. 2018a).

Intermediate-duration oral studies observed hepatic effects in various mammal species at doses ranging from 1.6 to 199 mg Cu/kg/day, primarily in rats (Epstein et al. 1982; Haddad et al. 1991; Hashish and Elgaml 2016; Khushboo et al. 2018; Kumar et al. 2015, 2016a, 2016b; NTP 1993; Rana and Kumar 1980; Sakhaee et al. 2012; Seven et al. 2018; Tian et al. 2019) and mice (Kvietkauskaite et al. 2004; Sakhaee et al. 2014; Wu et al. 2020). Hashish and Elgaml (2016) reported hepatic effects in rats at 1.6 mg Cu/kg/day. The study primarily tested the effects of curcumin as protective against copper sulfate exposure, so only one copper sulfate dose was tested. Liver histopathology showed acute swelling of hepatocytes, a few of which showed coagulative necrosis represented by karyolysis of nuclei, and mild hyperplasia of the epithelium lining of bile ducts with the presence of newly formed bile ductules. Further, hepatic damage due to membrane damage was indicated by elevated levels of hepatic marker enzymes of AST, ALT, ALP, and GGT. Lipid damage was indicated by increased MDA and decreased hepatic catalase, SOD, and GSH (Hashish and Elgaml 2016).

Changes in liver enzyme activity, an early sign of copper liver toxicity, appear to correlate with increased copper concentrations in the liver following oral exposures (Epstein et al. 1982; Kumar et al. 2016a, 2016b; Sakhaee et al. 2012; Tian et al. 2019). In Sprague-Dawley rats given 8.6 mg Cu/kg/day in water, liver copper concentrations and AST increased after 90 days, indicating liver cell damage (Epstein et al. 1982). Sakhaee et al. (2012) did not observe a clear toxicological effect of copper on serum AST and ALT over 56 days in rats exposed to 39.8 mg Cu/kg/day. However, at the higher dose of 79.6 mg Cu/kg/day, AST and ALT increased substantially with time, indicating a dose-response relationship (Sakhaee et al. 2012). In Wistar rats copper sulfate was administered via gavage daily for 30, 60, or 90 days, and a dose-response and duration-related increase of ALT was noted, along with duration-related increases in AST and bilirubin after administration of 25.5–50.9 mg Cu/kg/day (Kumar et al. 2015). Increased serum ALT and AST were reported in rats exposed to 39.8–79.6 mg Cu/kg/day for 30 days (Khushboo et al. 2018; Kumar and Sharma 1987; Tian et al. 2019) and 34 mg Cu/kg/day for 13 weeks (NTP 1993). Kumar et al. (2016b) found that increased ALT and AST correlated with decreased GSH and total antioxidant capacity, and with increased MDA and copper concentrations in liver tissue. These effects were found to be dose- and exposure duration-dependent.

Several studies reported histological findings in the liver at doses higher than the dose tested in Hashish and Elgaml (2016). The livers of male albino rats showed centrilobular necrosis and perilobular sclerosis with nuclear edema following a 20-day exposure to 39.8 mg Cu/kg/day as copper sulfate in the diet (Rana and Kumar 1980). At the same dose, an 8-week study in Wistar male rats reported hepatic lesions characterized by hepatocyte cell swelling, centrilobular necrosis, mild bile retention, and the presence of apoptotic bodies (Sakhaee et al. 2012). 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 for 30 days (Khushboo et al. 2018). However, Abe et al. (2008) found no significant difference in liver weight in Fischer 344 rats receiving 62 mg Cu/kg/day for 6 weeks compared to controls. Pregnant Wistar rats exposed to 130 mg Cu/kg/day prior to mating and during gestation (up to 73 days total) showed histopathological changes in the liver including hypertrophy and degeneration of hepatocytes and areas of focal necrosis (Haddad et al. 1991). Seven et al. (2018) reported hepatocellular degeneration and necrosis, and karyolysis and karyomegaly in some hepatocytes with a dose of 199 mg Cu/kg/day in male Sprague-Dawley rats. Increased MDA and decreased GSH, SOD, and catalase were also noted.

NTP (1993) conducted a 13-week study in rats 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, 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 from 31-285 mg Cu/kg/day (NTP 1993). The 15-day NTP animal studies tested lower doses in both sexes but did not evaluate serum chemistry changes. Kumar et al. (2016a) noted dose- and duration-related increases in the severity of histological findings in rats. Findings in the liver tissue included massive fatty liver change and centrilobular necrosis. Some rats showed occasional cell necrosis and petechial hemorrhage; mononuclear cell infiltration indicated hepatitis.

There is some evidence of a dose-response relationship between copper dose and hepatic effects in mice. No effect was seen in liver tissue following exposure to oral doses of 0.4, 1, or 2 mg Cu/kg/day; however, at 4 mg Cu/kg/day, mice showed lower hepatic cellularity and liver hemorrhage (Kadammattil et al. 2018). This finding is supported by observations of granular and vacuolar degeneration in hepatocyte and increased rate of hepatic apoptosis in mice exposed to 4 mg Cu/kg/day for 42 days (Wu et al. 2020). At 5.6 mg Cu/kg/day, mice showed decreased total liver protein, and mice exposed to 10.7 mg Cu/kg/day had significantly reduced liver weight along with increased liver copper levels (Kvietkauskaite et al. 2004). Similar to rats, there were significant increases in AST, ALT, and liver copper concentrations in mice at 79.6 mg Cu/kg/day (Sakhaee et al. 2014). In the NTP (1993) studies, no hepatic effects were seen in female mice exposed to 36 or 781 mg Cu/kg/day in either water or feed, respectively, for 15 days or

following 13-week exposures to doses ≤1058 mg Cu/kg/day. Similarly, no hepatic effects were seen in male mice exposed to 24 or 197 mg Cu/kg/day in either water or feed, respectively, for 15 days or following 13-week exposures to doses ≤815 mg Cu/kg/day (NTP 1993).

Two out of six pigs fed a diet containing 16.5 mg Cu/kg/day 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). White Chinese big-ear rabbits showed increases in serum low-density lipoprotein and substantial decreases in triglycerides and very low-density lipoprotein; low-density lipoproteins are produced by the liver, while some triglycerides are from diet (Shen et al. 2005). In pregnant rabbits, post-mortem evaluations found no exposure-related changes in the liver or gallbladder following daily oral exposure to 7.5-30 mg Cu/kg/day, as copper hydroxide, from gestation days 7 to 28 (Munley 2003a). A similarly designed study observed pale liver in animals that died from exposure to 18 mg Cu/kg/day (Munley 2003b). In infant guinea pigs exposed to 9.6 mg Cu/kg/day in water after weaning, no liver histological or organ weight changes were observed (Seffner et al. 1997).

Dermal

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).

No evidence of exposure-related gross lesions or organ weight difference were observed in the liver of rats dermally exposed to 0, 9, 36, or 181 mg Cu/kg, as copper 8-quinolinolate, on shaved back skin for 6 hours/day, 5 days/week for 4 weeks (Hagemann 1992).

Other Routes

Hepatic effects have been observed in humans following intentional injection of copper substances in two cases. 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 (typically used in cattle) and then developed acute hepatic failure with changes in liver enzymes, including 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).

2.10 RENAL

Inhalation

Data regarding renal toxicity of copper inhalation in humans is limited to a single case study. A two-year-old female child 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).

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

Oral

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 2010; Lubica et al. 2017; Malik and Mansur 2011; Mortazavi and Jafari-Javid 2009; 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. 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).

Several experimental rat studies confirm that the kidney is a target of copper toxicity in cases of copper overload. The mechanisms for copper renal toxicity are reported to be similar to those seen in the liver. Copper ions are a catalyst for initiating oxidative stress by generating reactive oxygen species or inhibiting enzyme activity (Abuja and Albertini 2001; Musacco-Sebio et al. 2017). Hashish and Elgaml (2016) reported indicators of renal toxicity that occurred with histological changes and biochemical changes in the kidneys of female rats given as low as 1.6 mg Cu/kg/day as copper sulfate in feed for 30 days. Kidney function was impaired as indicated by increased urea and creatinine levels in the serum,

indicating that the kidney was unable to filter and remove these reaction byproducts from the blood (Hashish and Elgaml 2016). Additionally, copper caused significant decreases in renal SOD, GSH, and catalase activity. Lipid peroxidation was further indicated by significantly increased MDA, which is an end-product of lipid peroxidation. Study authors suggest that lipid peroxidation may play a role in copper-induced renal toxicity (Hashish and Elgaml 2016). Histological examination of the kidneys noted degeneration of renal tissues and degeneration of the epithelial lining of some renal tubules.

No renal effects were observed in other species besides rats. 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. NTP (1993) reported no renal effects in male and female mice exposed to 24-36 mg Cu/kg/day and 717-781 mg Cu/kg/day for 15 days in water and feed, respectively, and in males and female exposed for 13 weeks to 815–1,058 mg Cu/kg/day in feed. No renal effects were reported in guinea pigs exposed to 6.6 mg Cu/kg/day for 4 weeks pre-weaning followed by 9.6 mg Cu/kg/day for up to 6 months (Seffner et al. 1997).

Several studies reported that copper concentration in the kidneys is proportional to the exposure dose and duration (Kumar et al. 2015, 2016a, 2016b). Kidney dysfunction indicated by significantly elevated blood urea nitrogen (BUN) was observed at 17 mg Cu/kg/day for 13 weeks (NTP 1993), 25.5 mg Cu/kg/day for 90 days (Kumar et al. 2015), and 39.8 mg Cu/kg/day for 8 weeks (Sakhaee et al. 2012). Kumar and Sharma (1987) reported significantly elevated urea levels at 39.8 mg Cu/kg/day for 30 days as an indicator of kidney damage. Increased BUN and serum creatinine correlated positively with free copper levels and hepatic MDA and inversely with GSH and total antioxidant capacity (TAC). The severity of effects increased with the duration of exposure (Kumar et al. 2016b). A second study by Kumar et al. (2016a) reported that the histopathological grading score (measure of severity) correlated positively with BUN and renal MDA levels and correlated inversely with GSH and TAC levels. Elevated MDA and reduced GSH are indicators of inflammatory renal injury.

Copper-induced histological changes in the kidneys of the male rats exposed to 39.8 mg Cu/kg/day as copper sulfate for 20–90 days included necrosis of the tubules, engorgement of uriniferous tubules, nuclear pyknosis, and cell proliferation in the medullary region, hemorrhage, and degeneration of Bowmen’s capsule in the cortex (Kumar et al. 2016a; Rana and Kumar 1980). A 15-day study in rats reported protein droplets in epithelial cells of the proximal tubule following exposure to 10 mg Cu/kg/day as copper sulfate (NTP 1993). The severity of histological findings increased with dose and exposure duration. Kumar et al. (2016a) observed increasing histological damage scores in rats treated for 30, 60, or 90 days, and in rats treated with a higher dose of copper sulfate. The grading criteria used a 1-5 scale to

grade vascular, inflammatory, and cellular degenerative changes in the kidney (Kumar et al. 2016a). Significantly increased urea and creatinine were noted in rats that showed bilateral enlargement of the kidney combined with a dark brown tissue color following 30 days of exposure to 50.9 mg Cu/kg/day as copper sulfate pentahydrate (Khushboo et al. 2018). Sakhaee et al. (2012) reported decreased BUN in rats treated with 39.8 mg Cu/kg/day and observed renal lesions with mild tubular necrosis and hyaline casts in renal tubules following 8 weeks of exposure. Similar histological observations included an increase in cortical tubule protein droplets, destroyed glomeruli corpuscles, plus damage to the epithelial lining of the proximal and distal convoluted tubules in rats exposed to doses ranging from 93–199 mg Cu/kg/day (Alharbi et al. 2019; NTP 1993; Seven et al. 2018).

Histopathological changes occurred in the kidneys of pregnant rats following exposure to 130 mg Cu/kg/day before and during gestation (Haddad et al. 1991). Changes included cloudy swelling of the proximal convoluted tubules due to hydropic degeneration and obliteration of the lumen. As noted with lower doses, similar changes in MDA, GSH, SOD, and catalase levels were observed at higher doses after 21 days of exposure (Seven et al. 2018). In Alharbi et al. (2019), there were similar effects on serum urea, creatinine, MDA, and GSH noted following a 7-day exposure. NTP (1993) reported no renal effects in female rats exposed to 31–285 mg Cu/kg/day for 15 days, and in male rats exposed to 46 or 140 mg Cu/kg/day in feed for either 15 days or 13 weeks, respectively. Alharbi et al. (2019) reported that high doses of copper (119 mg Cu/kg/day) in rats increased DNA fragmentation in kidney tissues, along with other previously noted histological changes. The authors suggested that copper induces apoptosis of renal tubules via activation of Bcl-2 associated X protein and suppression of Bcl-2. Alharbi et al. (2019) showed that copper as copper sulfate decreased expression levels of Bcl-2.

Of two pregnant rabbits that died during exposure to 30 mg Cu/kg/day from gestation days 7 to 28, one rabbit's death was attributed to a hemolytic event causing hemoglobin nephropathy and likely renal failure (Munley 2003a). The other dead rabbit had an autolyzed small liver. No effects were seen in kidneys of pregnant rabbits exposed to doses ≤18 mg Cu/kg/day (Munley 2003a, 2003b).

Dermal

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

No evidence of exposure-related gross lesions was observed in the kidneys or urinary bladder of rats dermally exposed to 0, 9, 36, or 181 mg Cu/kg, as copper 8-quinolinolate, on shaved back skin for 6 hours/day, 5 days/week for 4 weeks (Hagemann 1992). Additionally, no differences in renal function parameters, including the concentrations of urea and creatinine in urine, were seen.

2.11 DERMAL

Inhalation

Information regarding dermal effects in humans following copper inhalation are limited to one occupational study and two case studies, both of which involved simultaneous inhalation and dermal exposures. 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. 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 concentrations ranging from 111 to 464 mg Cu/m^3 over a 3-year period (Suciu et al. 1981). For reference, the PEL by OSHA for an 8-hour TWA exposure to copper dusts in general industry is 1 mg/m^3 (OSHA 2020a).

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

Oral

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).

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

Dermal

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, this may be due to the substance being hot (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).

New Zealand white rabbits exposed to 160 mg Cu/kg, as a copper herbicide, for 24 hours showed erythema, edema blanching, eschar, desquamation, and exfoliation at the site of application (Rush 1990a). However, no control was tested to determine if effects resulted from the copper. No evidence of exposure-related skin irritation at the application site was seen in rats dermally exposed to 0, 9, 36, or 181 mg Cu/kg, as copper 8-quinolinolate, on shaved back skin for 6 hours/day, 5 days/week for 4 weeks (Hagemann 1992).

Other Routes

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). A 29-year-old pregnant woman developed cyanosis after intentionally vaginally inserting an unknown amount of copper sulfate powder diluted in water (Motlhatlhedi et al. 2014).

2.12 OCULAR

Inhalation

No studies were located regarding ocular effects in human or animals following inhalation exposure to copper.

Oral

Data regarding ocular effects in humans following ingestion of copper is limited to one case study where a 17-year-old boy developed yellowing of his sclera after ingesting 10 g copper sulfate (Du and Mou 2019). This case also presented with light yellowing of the skin and elevated total bilirubin.

Data in animals is limited to one study where histopathological observations in rats exposed to 50.9 mg Cu/kg/day for 30 days revealed paleness of mucous membranes of eyes (Khushboo et al. 2018).

Dermal

Eye irritation has been 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 three 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 into his eye, causing the ulcer and vision loss.

No evidence of exposure-related ocular effects was seen in rats dermally exposed to 0, 9, 36, or 181 mg Cu/kg, as copper 8-quinolinolate, on shaved back skin for 6 hours/day, 5 days/week for 4 weeks (Hagemann 1992). The eyes were examined for clinical signs throughout the exposure period.

2.13 ENDOCRINE

Inhalation

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 toxicokinetics (Suciu et al. 1981); however, both the significance of this effect and its relationship to copper exposure cannot be determined.

Oral

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 33-year-old woman developed adrenal insufficiency with reduced cortisol after intentionally ingesting an unknown amount of copper sulfate (Sinkovic et al. 2008). A 53-year-old man developed acute pancreatitis after intentionally ingesting 120 g copper sulfate well above reported lethal doses; medical intervention prevented death (Lubica et al. 2017). In all cases, the endocrine effects were not permanent, and the patients made full recoveries within weeks.

Following histological analysis and weighing of the adrenal glands, no toxic effects were identified in young guinea pigs exposed to 9.6 mg Cu/kg/day starting on the first or second day of life and persisting for 6 months (Seffner et al. 1997). No histological differences were observed in the adrenal, mammary, parathyroid, pituitary, preputial, or clitoral glands of rats exposed to doses as high as 31–36 mg Cu/kg/day in water or 285–325 mg Cu/kg/day in feed for 15 days, or to dietary doses of up to 134-140 mg Cu/kg/day for 13 weeks (NTP 1993). Similarly, no difference in these measures were seen in mice exposed to doses as high as 24-62 mg Cu/kg/day for 15 days in water, or to doses as high as 815–1,058 mg Cu/kg/day for 13 weeks in their diet (NTP 1993). In pregnant rabbits, postmortem evaluations found no exposure-related changes in the pancreas following daily exposure to 7.5–30 mg Cu/kg/day as copper hydroxide from gestation days 7 to 28 (Munley 2003a)

Dermal

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

No evidence of exposure-related gross lesions was observed in the adrenal, pituitary, or thyroid glands or in the thymus of rats dermally exposed to 0, 9, 36, or 181 mg Cu/kg, as copper 8-quinolinolate, on their shaved back skin for 6 hours/day, 5 days/week for 4 weeks (Hagemann 1992). Additionally, there were no organ weight differences in the adrenals or thymus gland; the pituitary gland was not weighed.

2.14 IMMUNOLOGICAL

Inhalation

A single study in humans exposed to copper-containing welding fumes reported asymptomatic systemic inflammation evidenced by a significant increase in blood C-reactive protein (Markert et al. 2016). Men were exposed to a copper concentration of 0.53 mg/m^3 in welding fume for 6 hours per period, for 3 periods over 3 weeks. Welding fumes were generated in a separate room and were connected by a ventilation system to the room where subjects were exposed. The C-reactive protein increase was the highest during the period between the 6th and 24 hours after exposure ended (Markert et al. 2016).

An acute exposure study in mice reported an impaired immune response following inhalation exposure to copper sulfate after presentation of a bacterial challenge (Drummond et al. 1986). In CD-1 mice challenged by an aerosol of *Streptococcus zooepidemicus,* decreased pulmonary macrophage bactericidal activity was observed in those exposed to 0.56 mg Cu/m^3 for 3 hours and in those exposed to 0.12 mg Cu/m^3 for 3 hours/day, 5 days/week for 2 weeks. In the same study, bactericidal activity of alveolar macrophages decreased in mice exposed to 3.3 mg Cu/m^3 for 3 hours or 0.13 mg Cu/m^3 for 3 hours/day, 5 days/week for 2 weeks after exposure to an aerosol of *Klebsiella pneumonia* (Drummond et al. 1986). In a different study, there were no functional differences in macrophages in rabbits exposed to 0.28 mg Cu/m^3 for 6 hours/day, 5 days for 1 month (Johansson et al. 1983).

Oral

Three studies examined immunological effects in humans following copper ingestion. Nine men were studied for immune effects following copper exposure in a metabolic research unit during two separate 18-day study periods (Turnlund et al. 2004). During the first 18-day exposure to 0.02 mg Cu/kg/day in diet, no effects were seen on white blood cells, lymphocytes, immunoglobin, interleukin 2R, or interleukin 6. During the second 18-day period of exposure to 0.1 mg Cu/kg/day, the men had significant changes in lymphocyte levels and significantly less antibody to an influenza strain they were immunized

for, compared to the controls who were also immunized (Turnlund et al. 2004). Reduced ALB and GLB were observed in a 17-year-old boy who ingested 10 g copper sulfate (Du and Mou 2019). In the second study, among 29 patients with chronic copper poisoning from plumbing, one had a natural-killer cell deficiency (Eife et al. 1999). Copper levels in tap water measured in homes of these patients ranged from 0.1 to 16.9 mg/L.

Studies on immunotoxicity following oral exposure to copper in animals are limited to a few studies examining the spleen. In mice, no effects of immunotoxicity were seen following a daily 7-day exposure to 2 mg Cu/kg/day; however, follicular hyperplasia in the spleen was observed at 4 mg Cu/kg/day (Kadammattil et al. 2018). Rats exposed for 30 days to 50.9 mg Cu/kg/day showed congested and enlarged spleens (Khushboo et al. 2018). No histopathological changes were seen in the spleens of rats exposed to doses as high as 31–36 mg Cu/kg/day in drinking water or as high as 285–325 mg Cu/kg/day in feed for 15 days (NTP 1993). There were no changes in the spleen of young guinea pigs following exposure to 9.6 mg Cu/kg/day for 6 months (Seffner et al. 1997).

One study in mice exposed to 5.6 mg Cu/kg/day showed that exposure had a toxic effect on antioxidant defense system enzymes and phenotypic properties of immunocompetent cells of the mice as evidenced by decreases in percentage of suppressor, natural killer, and precursor cells and increases in immunoregulatory index (Kvietkauskaite et al. 2004). In rats exposed to 199 mg Cu/kg/day for 21 days, serum tumor necrosis factor-alpha (TNF) levels were increased 1.55 times greater than in controls (Seven et al. 2018). In pregnant rabbits, postmortem evaluation found no exposure-related changes in the spleen following daily exposure to 7.5–30 mg Cu/kg/day, as copper hydroxide, from gestation days 7 to 28 (Munley 2003a).

Dermal

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). Axillary lymphadenopathy was reported in an 11-year-old boy who had copper sulfate crystals intentionally applied to his hands (Lapid 2008).

Five of five male rats exposed to 181 mg Cu/kg, as copper 8-quinolinolate, had higher incidence of necrotic thymic lymphocytes (Hagemann 1992). No evidence of exposure-related gross lesions was observed in the lymph nodes of male and female rats dermally exposed to 0, 9, 36, or 181 mg Cu/kg, as

copper 8-quinolinolate, on shaved back skin for 6 hours/day, 5 days/week for 4 weeks (Hagemann 1992). Additionally, there was no significant change in spleen weight.

2.15 NEUROLOGICAL

Inhalation

Three studies evaluating neurological effects in humans following copper inhalation were located. Headache, vertigo, and drowsiness were reported in factory workers exposed for 3 years, beginning with a max concentration of 464 mg/m^3 and declining over 3 years to 111 mg/m^3 copper dust (Suciu et al. 1981). A 2-year-old girl who accidentally inhaled copper dust that also got on her facial skin experienced sensory impairment (Donoso et al. 2007). An epidemiological study on children aged 8 to 12 years found that airborne copper exposure was significantly associated with poorer motor performance and detectable signs of brain damage (Pujol et al. 2016). Copper levels, primarily attributed to traffic pollution, were measured inside and outside of participant schools; 2,827 children participated in behavioral testing and a subset of 263 children participated in brain imaging. The reaction time in children with higher exposure was reduced, while imaging showed copper associated with higher gray matter concentration in the striatum. Copper also appeared to be related to changes in the architecture of neural tissue diffusion (Pujol et al. 2016). Additional details on this study are in Table 2-4.

Oral

Seven adult females exposed to 0.07 mg Cu/kg/day for 2 weeks resulted in clinical symptoms and increased salivation in six of the subjects and headaches in all subjects (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 other cases. 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).

No histological changes were observed in the brains of mice exposed to 4 mg Cu/kg/day for 7 days (Kadammattil et al. 2018). In mice exposed to trace levels of copper in drinking water (0.08 mg Cu/kg/day) for 21 days, there was no significant change in learning or memory performance as tested by the step-through tasks and Morris water maze task (Lu et al. 2009). At higher doses in mice, females had a 10% increase in relative brain weight following 13 weeks of exposure to 267 mg/Cu/kg/day, and males had a 13% increase in relative brain weight (NTP 1993). In the NTP (1993) study, no neurological effects were seen following exposure to doses of 44-97 mg Cu/kg/day or 52-267 mg/Cu/kg/day in the males and females, respectively (NTP 1993). Additionally, in the shorter duration 15-day mouse study, no

neurological effects were reported in the males receiving 10 to 58 mg Cu/kg/day/day or the females receiving 15 to 62 mg Cu/kg/day (NTP 1993). Although doses higher than 58 and 62 mg Cu/kg/day were tested, the mortality was high, reducing the ability to quantitatively evaluate statistical significance as compared to controls. Seffner et al. (1997) found no significant differences in brain weight of young guinea pigs exposed to 6.6 mg Cu/kg/day starting 1-2 days after birth and continuing through 4 weeks of preweaning followed by an increase to 9.6 mg Cu/mg/day for up to 6 months. Weakness preceded death in one pregnant rabbit that died from exposure to 18 mg Cu/kg/day from gestation days 7-18 (Munley 2003b).

Multiple neurological effects were observed in rats exposed to doses >4 mg Cu/kg/day for intermediate exposure durations. The lowest LOAEL for neurological effects was 4 mg Cu/kg/day in rats that showed neurobehavioral changes (Kumar et al. 2019). Changes include 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 entries in an open-arm test, and decreased exploration time (Kumar et al. 2019). 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). A feeding study in rats exposed to 250 ppm copper in the diet (daily dose could not be calculated) as copper sulfate reported no effects on spontaneous motor activity (assessed using an actophometer), learning ability (assessed using a pole climbing chamber), or relearning capacity and memory (assessed using a Y-maze) (Murthy et al. 1981). The same study observed 16% and 17% decreases in dopamine and norepinephrine neurotransmitters, respectively. 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 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, plus an increase in resting time (Kalita et al. 2020; Kumar et al. 2015). Cell death through apoptosis and inflammatory pathways were indicated by increased expression of glial fibrillary acidic protein (GFAP) and caspase-3 (Kalita et al. 2020).

Multiple studies have demonstrated that copper, as copper sulfate, induces oxidative stress in the brain as evidenced by reduced SOD, catalase activity, TAC, and GSH, accompanied by increased brain MDA (Kumar et al. 2016b, 2019). 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). This was based on changes in grip strength, plus rotarod, and Y-maze tests results 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 authors (Kumar et al. 2016a). Histological findings graded on a 1 to 5 severity scale were positively correlated with MDA levels in the brain and inversely correlated with TAC and GSH levels (Kumar et al. 2016a).

In a separate study by Behzadfar et al. (2017), rats were exposed daily for 21 days starting at doses ≥39.8 mg Cu/kg/day. Dose-dependent reactive oxygen species (ROS) formation was demonstrated by increased hippocampal mitochondrial MDA formation (52%) and decreased mitochondrial GSH (29%). Mitochondrial swelling, an indicator of mitochondrial membrane permeability, was also dose dependent. Additionally, spatial memory of rats was tested using the Morris water maze task, which revealed that the impairment was dose dependent, as escape latency was significantly increased at 39.8 mg Cu/kg/day and distance traveled was increased at 79.6 mg Cu/kg/day. The study reported a NOAEL of 19.9 mg Cu/kg/day for neurotoxicity. The study authors suggested that ROS generation in hippocampal mitochondria due to copper exposure caused memory impairment (Behzadfar et al. 2017).

A study that only tested one dose (50.9 mg Cu/kg/day) in rats for 30 days reported that copper toxicity slowed brain activity and produced a swollen, congested, and edematous brain (Khushboo et al. 2018). Gliosis occurred in the brain of 10/10 female rats exposed to 134 mg Cu/kg/day in their diets for 13 weeks (NTP 1993). In the same study, males exposed to 140 mg Cu/kg/day had a 27% increase in relative brain weight at necropsy compared to controls. No neurological effects were seen at doses of 9–68 mg Cu/kg/day in females and 8–66 mg Cu/kg/day in males (NTP 1993). In the 15-day study, no neurological effects were seen in rats exposed to copper in water or feed at doses of 10–325 mg Cu/kg/day and 10–285 mg Cu/kg/day in males and females, respectively (NTP 1993).

Dermal

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

No evidence of exposure-related gross lesions was observed in the peripheral nerve, brain, or spinal cord of rats dermally exposed to 0, 9, 36, or 181 mg Cu/kg, as copper 8-quinolinolate, on shaved back skin for

6 hours/day, 5 days/week for 4 weeks (Hagemann 1992). Additionally, no differences in brain weight were seen.

Other Routes

A 29-year-old pregnant woman who intentionally vaginally inserted an unknown amount of copper sulfate diluted in water was reported to be cyanotic with a decreased consciousness 12 hours after admission for copper poisoning (Motlhatlhedi et al. 2014). The patient was only responding to pain.

2.16 REPRODUCTIVE

Inhalation

Sexual impotence was reported in 16% of workers (75–100 workers examined) exposed to 111– 464 mg/m^3 copper dust during grinding and sieving operations (Suciu et al. 1981). The significance of this finding is difficult to assess because the study did not include a control group.

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

Oral

No studies were located regarding reproductive effects in humans following oral exposure to copper. Two cross-sectional studies (described in detail in Table 2-7) reported associations between serum copper levels, presumably resulting from oral ingestion, and reproductive outcomes (De Craemer et al. 2017; Kasperczyk et al. 2016). Due to usual limitations of cross-sectional studies, the results cannot be definitively attributed to copper exposure or blood copper levels.

Many animal studies have examined the reproductive toxicity of copper following oral exposures. Few of those studies examined female reproductive toxicity (Babaei et al. 2012; Munley 2003a, 2003b; NTP 1993). Female mice were exposed to 39.8 or 79.6 mg Cu/kg/day for either 14 or 35 days (Babaei et al. 2012). At both exposure durations serious effects occurred at 39.8 mg Cu/kg/day including a decrease in the number of antral follicles, ovarian cell damage, and a decrease in the corpus luteum. The severity of the antral follicle effects increased with dose. There were also decreases in the numbers of other follicles (i.e., primordial, primary, growing, and secondary), accompanied by severe changes to follicle structure. A 13-week NTP (1993) study in female rats reported chronic active inflammation of the clitoral gland and ovarian cysts in 10/10 rats exposed to 134 mg Cu/kg/day. However, no effects were seen in lower doses

of 9–68 mg Cu/kg/day. The 13-week study in female mice reported cysts in the clitoral glands of 8/10 female mice exposed to 1,058 mg Cu/kg/day (NTP 1993). No effects were seen in the female mice exposed to 52–536 mg Cu/kg/day. In 15-day studies, no histological or vaginal cytology alterations were reported in female rats exposed to 10–285 mg Cu/kg/day or in mice exposed to 15–36 mg Cu/kg/day (NTP 1993). In pregnant rabbits exposed daily to 30 mg Cu/kg/day as copper hydroxide from gestation days 7 to 28 there was an increase in fetal resorptions compare to controls (Munley 2003a). Total mean resorptions per litter were 1.3 for the 30 mg Cu/kg/day dose group compared to 0.3 resorptions for controls. At a dose of 15 mg Cu/kg/day no reproductive differences were observed in the rabbits (Munley 2003a). At the same exposure duration and frequency, two pregnant rabbits aborted pregnancies on gestation day 22 after exposure to 18 mg Cu/kg/day (Munley 2003b).

Multiple studies in male rats and mice exposed to copper for intermediate durations suggest that copper plays a role in spermatogenesis and male infertility. In a study exposing an unspecified number of male mice to 4 mg Cu/kg/day as copper sulfate, the frequency of folded sperm increased but other indicators of abnormal sperm morphology were not significantly altered (Kadammattil et al. 2018). Significant decreases in sperm concentration, count, motility, and viability were reported in rats exposed to ≥39.8 mg Cu/kg/day for 30 days–56 days (Liu et al. 2016; Sakhaee et al. 2012) and in mice exposed to ≥39.8 mg Cu/kg/day once every 2 days for 28-42 days or daily for 42 days (Sakhaee et al. 2016a, 2016b). In rats dosed at 39.8 mg Cu/kg/day, there were decreases in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Liu et al. 2016). In mice, histological observations included depletion and vacuolation of seminiferous epithelium and degeneration of the seminiferous tubules at the same dose (Sakhaee et al. 2016a, 2016b). The severity of reproductive toxicity in male animals was found to be dose dependent (Liu et al. 2016; Sakhaee et al. 2012). The signs of reproductive toxicity reported at 39.8 mg Cu/kg/day were also present in two studies that only tested a dose of 50.9 mg Cu/kg/day in rats for 30 or 90 days (Arafa et al. 2019; Khushboo et al. 2018). 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). Khushboo et al. (2018) suggests an association between excess oral copper exposure and decreased synthesis of testosterone. Arafa et al. (2019) had similar findings, and study authors linked this to the downregulation of AT1R and ACE1 protein levels in testicular tissues. An in vitro study of rabbit spermatozoa exposed to copper sulfate found sperm abnormalities including altered anterior part of the sperm head and in the connection segment (Roychoudhury et al. 2010).

As previously mentioned, Liu et al. (2016) reported that several markers of male reproductive toxicity were dose dependent in rats. Additionally, at the highest dose tested (79.6 mg Cu/kg/day) in their study, 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 but not after 28 days of exposure. In two studies, male mice exposed to 79.6 mg Cu/kg/day had 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). Intermediate-duration studies by NTP (1993) found a significant 27% increase in relative right testis weight in male rats exposed to 140 mg Cu/kg/day for 13 weeks compared to controls; however, no histological or sperm morphology alterations were seen in male rats exposed to 66 mg Cu/kg/day. In male mice exposed to 187 mg Cu/kg/day, relative right testis weight was 12% greater than controls but no histological changes in the reproductive system or sperm morphology alterations were seen in mice exposed to doses up to 398 mg Cu/kg/day (NTP 1993).

Dermal

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

There was no evidence of exposure-related gross lesions in the seminal vesicle, testis, or epididymis of male rats, or in the uterus, vagina, or ovaries of female rats. Dermal exposures in both the male and female rats were 0, 9, 36, or 181 mg Cu/kg, as copper 8-quinolinolate, applied to shaved area on their backs for 6 hours/day, 5 days/week for 4 weeks (Hagemann 1992). Additionally, there were no exposure-related differences in organ weights for the ovaries of females or the testes of males.

Other

A 29-year-old pregnant woman who intentionally vaginally inserted an unknown amount of copper sulfate powder diluted in water experienced a blue vaginal discharge, blue colored mucous on the cervix surface, and vaginal loss of amniotic fluid (Motlhatlhedi et al. 2014).

2.17 DEVELOPMENTAL

Inhalation

No toxicity studies were identified for developmental effects in humans and animals following inhalation exposure to copper. A retrospective birth cohort study, described in Table 2-4, did not report any associations of copper in the inhaled particulate matter with low birth weight (Pedersen et al. 2016).

Oral

No studies were located regarding developmental effects of humans following oral exposure to copper. A case-control study of mothers who had given birth to children with neural tube defects did not find any difference in the mean copper levels reported for their drinking water compared to mothers of children without neural tube defects (Longerich et al. 1991).

Data on the developmental toxicity of copper in experimental animals is limited. No significant difference was reported for the number of implantations, non-viable embryos, resorbed embryos, or mean embryo weight from pregnant mice exposed to 4 mg Cu/kg/day for 7 days from the 7^{th} to 12^{th} days of pregnancy as compared to controls (Kadammattil et al. 2018). After pregnant rats were exposed to 130 mg Cu/kg/day in diet before and during gestation, their offspring showed delayed growth and development (Haddad et al. 1991). In 11.5-day-old embryos, significant decreases in mean somite number, crown-rump length, and yolk sac diameter were observed. In 21.5-day-old fetuses and newborns, delayed ossification was observed in the cervical and cauda vertebrae, sternum, metacarpals, forelimb phalanges, metatarsals, and hindlimb phalanges (Haddad et al. 1991).

Pregnant rabbits were exposed to 0, 7.5, 15, or 30 mg Cu/kg/day, as copper hydroxide, from gestation days 7 to 28. The fetuses were removed and examined on gestation day 29 (Munley 2003a). Those exposed to the highest dose had 12% reduced mean weight compared to controls. Four of the fetuses were observed to have protrusion of intestines at the umbilicus (omphalocele) (Munley 2003a). In a similarly designed study, maternal exposure to 18 mg Cu/kg/day resulted in significantly increased incidence of hemivertebra, delayed ossification, and supernumerary ribs when compared to the controls (Munley 2003b).

Dermal

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

2.18 OTHER NONCANCER

Inhalation

A few studies have reported metal fume fever, a 24–48-hour illness characterized by chills, fever, aching muscles, dryness in the mouth and throat, and headache, in workers exposed to copper dust or fumes (Armstrong et al. 1983; Gleason 1968). Gleason (1968) reported airborne copper dust concentrations of 0.075–0.12 mg/m^3. This range is lower than the OSHA PEL of 1 mg/m^3 for an 8-hour TWA exposure to copper dusts in general industry. It has been suggested that other metals present in the workplace could have been the causative agent for the metal fume fever, rather than copper. A review by Borak et al. (2000) supports this hypothesis as it reviewed occupational reports of metal fume fever and concluded that there is insufficient evidence to suggests these were caused by copper fumes or dusts as other agents appeared to contribute to reported symptoms.

Oral

Several experimental studies reported various noncancerous effects in rats or monkeys. Male rats ingested kaolin indicating pica behavior following exposure once to 10 mg Cu/kg as copper sulfate pentahydrate (Yamamoto et al. 2004). In the same study, copper sulfate induced emesis (vomiting) in shrews but not pica behavior. Pregnant female rabbits exposed to 9 or 15 mg Cu/kg/day from gestation days 7-28 had a significant 17% and 22% reduction, respectively, in mean food consumption compared to controls (Munley 2003a). In rats, decreases in food consumption and water intake were attributed to oral exposure to 50.9-199 mg Cu/kg/day (Khushboo et al. 2018; Seven et al. 2018). Two chronic studies in monkeys reported no differences in food intake following chronic 3-year oral intakes of 0.77–1.05 mg Cu/kg/day from diet and supplements (Araya et al. 2012).

Dermal

No evidence of exposure-related changes in food intake was recorded in rats dermally exposed to 0, 9, 36, or 181 mg Cu/kg, as copper 8-quinolinolate, on shaved back skin for 6 hours/day, 5 days/week for 4 weeks (Hagemann 1992).

2.19 CANCER

Inhalation

There are limited data for humans and no data for animals on the carcinogenicity of inhaled copper. Although a number of studies examined cancer risk among workers at copper smelters, these papers are

not discussed because the cancer risk was attributed to arsenic exposure rather than to copper. In a study of over 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% confidence interval [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 were measured in the underground mines; between 1960 and 1990, radioactivity levels of $1.29x10^{-11}$ 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. Increases in lung cancer risk were observed in both groups, and 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. However, a prospective study of populations in Europe found that copper in $PM_{2.5}$ was associated with increased risk of lung cancer (Hazard Ratio (HR) = 1.25; 95% CI=1.01-1.53), while PM_{10} was not associated with increased risk of lung cancer (Hazard Ratio (HR) = 1.14; 95% CI=0.96-1.35) (Raaschou-Nielsen et al. 2016).

Oral

There is a case-control study that examined the relationship between copper loading in carpet dust, which can be ingested, inhaled, or can touch skin, and childhood acute lymphoblastic leukemia (Whitehead et al.

2015). There was no significant difference between the odds for cancer development between those exposed to high levels of carpet dust and those who were not.

Three oral studies examined the carcinogenicity of copper compounds in animals. 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 to weekly to 9 mg Cu/kg/day as an unspecified copper compound for 16 weeks (Greene et al. 1987). In a carcinogenicity study, rats were orally exposed to copper gluconate, 62 mg Cu/kg/day, for 6 weeks, and a significant increase in the number of glutathione *S*-transferase placental form (GST-P) positive single hepatocytes was seen compared to controls (Abe et al. 2008). However, the study authors remarked that the results are not necessarily indicative of carcinogenicity given that the induction of GST-P positive individual hepatocytes could act to protect the liver from exposure (Abe et al. 2008). The same study found evidence of carcinogenicity based on the increased number of GST-P positive lesions, given that injection with *N*-nitrosodiethylamine (DEN), a known carcinogen preceded the oral exposure to copper gluconate (Abe et al. 2008).

Dermal

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

Other

Several studies examined the carcinogenicity of copper compounds following parenteral administration. No clear tumor incidence results were observed in a collection of studies that used non-oral exposures, including 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); 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.

2.20 GENOTOXICITY

One study in humans examined the genotoxicity of copper following occupational exposure. Workers at a copper smelting plant showed significant increases in DNA damage of peripheral blood leukocytes

relative to controls. However, blood copper concentrations were not associated with the level of DNA damage (De Olivera et al. 2012). Shubber et al. (1998) analyzed blood lymphocytes of women using copper-containing contraceptive intrauterine devices (IUDs). Compared to age- and income-matched control women, those using IUDs had significantly higher 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 6 mg/day. 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; the results of these *in vivo* genotoxicity studies are summarized in Table 2-8. Significant increases in the occurrence of micronuclei and chromosomal aberrations have been observed in chick bone marrow cells and erythrocytes (Bhunya and Jena 1996) and mouse bone marrow cells following exposure to copper sulfate (Agarwal et al. 1990; Bhunya and Pati 1987; Fahmy 2000; Kadammattil et al. 2018; Prá et al. 2008). Rabbits treated with copper sulfate in drinking water showed significant increases in sister chromatid exchanges and chromosomal aberrations (Georgieva et al. 2013). A study of copper sulfate by Tinwell and Ashby (1990) did not find increases in the number of micronuclei in mouse bone marrow cells. Several studies reported DNA damage in blood cells of mice exposed to copper sulfate at doses ranging from 1.25–12.5 mg/kg (Franke et al. 2006; Saleha Banu et al. 2004; Prá et al. 2008). DNA fragmentation was also observed in liver cells of rats after oral exposures to 100 or 300 mg/kg/day of copper sulfate for 7 days (Alhusaini et al. 2018a, 2018b). In Drosophila, exposure to copper sulfate resulted in significant increases in the occurrence of recessive lethals (Law 1938) and DNA damage (Shukla et al. 2011). Sperm abnormalities, including spermatocyte chromosome aberrations, double-headed, and double-tailed sperm, were observed in mice after intraperitoneal exposure (Bhunya and Pati 1987; Fahmy 2000) and oral exposure to copper sulfate (Kadammattil et al. 2018).

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 (Garrett and Lewtas 1983; Sirover and Loeb 1976), oxidative DNA damage (Schwerdtle et al. 2007), and an increase in the occurrence of DNA strand breaks (Anchordoquy et al. 2017; Grillo et al. 2010; Jing et al. 2016; Schwerdtle et al. 2007; Sideris et al. 1988; Sina et al. 1983). Using a comet assay approach, DNA damage was detected in mouse lymphocytes from copper alone and in the presence of curcumin (Urbina-Cano et

al. 2006). Oxidative DNA damage repair was inhibited in human HeLa S3 cells following induction with visible light (Schwerdtle et al. 2007). Unscheduled DNA repair synthesis occurred in rat hepatocytes at copper concentrations of 7.9-78.5 µM, in the presence or absence of hydroxyurea (Denizeau and Marion 1989). An increase in sister chromatid exchange in Chinese hamster cells (Sideris et al. 1988) is consistent with the clastogenic effects observed in *in vivo* assays. Caicedo et al. (2008) found that copper at concentrations of 5 mM (318 mg/L) did not induce DNA damage in human CD4+ T lymphocytes, whereas Husain and Mahmood (2019) found DNA damage to human lymphocytes at copper concentrations of 2.5 mM (159 mg/L). No DNA damage was observed in human blood cells exposed to copper alone (Prasad et al. 2006). The results of *in vitro* genotoxicity studies are summarized in Table 2-9.

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). 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 in 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).

Table 2-8. Genotoxicity of Copper and Copper Compounds In Vivo

Species (test system)	Endpoint	Results	Reference	Compound
Non-mammalian cells:				
Drosophila melanogaster (oral exposure)	DNA damage	+	Shukla et al. 2011	Copper sulfate
Drosophila melanogaster (injection into larvae)	Recessive lethals	+	Law 1938	Copper sulfate
Mammalian cells:				
Albino rat liver cells (oral exposure)	DNA damage	+	Alhusaini et al. 2018a	Copper sulfate
Albino rat liver cells (oral exposure)	DNA damage	+	Alhusaini et al. 2018b	Copper sulfate

Table 2-8. Genotoxicity of Copper and Copper Compounds In Vivo

Species (test system)	Endpoint	Results	Reference	Compound
CF1 mice blood cells (gavage exposure)	DNA damage	+	Prá et al. 2008	Copper sulfate
Human leukocytes (oral exposure)	DNA damage	–	O'Connor et al. 2013	Copper
Human peripheral blood leukocytes	DNA damage	–	De Olivera et al. 2011	Copper
Swiss Albino mice leukocytes (oral exposure)	DNA damage	+	Saleha Banu et al. 2004	Copper sulfate
Swiss Webster mice blood cells (oral exposure)	DNA damage	+	Franke et al. 2006	Copper sulfate
Human blood leukocytes	Chromosomal aberrations	+	Shubber et al. 1998	Copper
Inbred Swiss mice bone marrow cells (intraperitoneal and/or subcutaneous injection)	Chromosomal aberrations	+	Bhunya and Pati 1987	Copper sulfate
New Zealand rabbit blood cells (oral exposure)	Chromosomal aberrations	+	Georgieva et al. 2013	Copper sulfate
White Leghorn chicken bone marrow cells (intraperitoneal injection and oral exposure)	Chromosomal aberrations	+	Bhunya and Jena 1996	Copper sulfate
White Swiss mice spermatocytes (intraperitoneal injection)	Chromosomal aberrations	+	Fahmy 2000	Copper sulfate
White Swiss mice (intraperitoneal injection)	Chromosomal aberrations	+	Agarwal et al. 1990	Copper sulfate
CBA mice bone marrow cells (intraperitoneal 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 (intraperitoneal and/or subcutaneous injection)	Micronuclei	+	Bhunya and Pati 1987	Copper sulfate
Swiss Albino mice bone marrow cells (oral exposure)	Micronuclei	+	Kadammattil et al. 2018	Copper sulfate
White Leghorn chicken bone marrow cells (intraperitoneal injection and oral exposure)	Micronuclei	+	Bhunya and Jena 1996	Copper sulfate
White Leghorn chicken erythrocytes (intraperitoneal injection and oral exposure)	Micronuclei	+	Bhunya and Jena 1996	Copper sulfate
White Swiss mice bone marrow cells (intraperitoneal injection)	Micronuclei	+	Fahmy 2000	Copper sulfate
Human blood leukocytes	Sister chromatid exchanges	+	Shubber et al. 1998	Copper
New Zealand rabbit blood cells (oral exposure)	Sister chromatid exchanges	+	Georgieva et al. 2013	Copper sulfate

Table 2-8. Genotoxicity of Copper and Copper Compounds In Vivo

Species (test system)	Endpoint	Results	Reference	Compound
Inbred Swiss mice (intraperitoneal 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 (intraperitoneal injection)	Sperm abnormalities	+	Fahmy 2000	Copper sulfate

+ = positive results; – = negative results; DNA = deoxyribonucleic acid

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

		Results			
Species (test system)	Endpoint	With activation	Without activation	Reference	Compound
Prokaryotic organisms:					
Avian myeloblasts virus, DNA polymerase	Errors in DNA synthesis	NT	+	Sirover and Loeb 1976	Copper chloride
Bacillus subtilis	Rec assay	NT	–	Nishioka 1975	Copper chloride
Salmonella typhimurium TA 102	Reverse mutation	NT	–	Marzin and Phi 1985	Copper sulfate
S. typhimurium TA98, TA102, TA1535, TA1537	Reverse mutation	–	–	Wong 1988	Copper chloride
S. typhimurium TA100	Reverse mutation	NT	–	Tso and Fung 1981	Copper chloride
Escherichia coli	Reverse mutation	NT	+	Demerec et al. 1951	Copper sulfate
Eukaryotic organisms:					
Fungi:					
S. cerevisiae	Recombination	NT	–	Sora et al. 1986	Copper sulfate
Saccharomyces cerevisiae	Reverse mutation	NT	–	Singh 1983	Copper sulfate
Mammalian cells:					
Human blood cells	DNA damage	NT	–	Prasad et al. 2006	Copper chloride
Human lymphocytes	DNA damage	NT	+	Husain and Mahmood 2019	Copper chloride
Human CD4+ T lymphocytes	DNA damage	NT	–	Caicedo et al. 2007	Copper
Human HeLa S3 cells	DNA strand breaks	NT	+	Schwerdtle et al. 2007	Copper sulfate
Human HeLa S3 cells	Impaired DNA damage repair	+	NT	Schwerdtle et al. 2007	Copper sulfate

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

Species (test system)	Endpoint	Results		Reference	Compound
		With activation	Without activation		
Human HeLa S3 cells	Oxidative DNA damage	NT	+	Schwerdtle et al. 2007	Copper sulfate
Chinese Hamster Ovary (CHO) cells	DNA strand breaks	NT	+	Grillo et al. 2010	Copper
Chinese Hamster Ovary (CHO) cells	DNA synthesis	NT	+	Garrett and Lewtas 1983	Copper chloride
Chinese hamster V79 cells	DNA strand breaks	NT	+	Sideris et al. 1988	Copper nitrate
Chinese hamster V79 cells	Sister chromatid exchange	NT	+	Sideris et al. 1988	Copper nitrate
Mouse Balb-C lymphocytes (comet assay)	DNA damage	+	+	Urbina-Cano et al. 2006	Copper
Mouse primary lymphocytes	DNA strand breaks	NT	+	Jing et al. 2016	Copper
Bovine ovary cells	DNA strand breaks	NT	+	Anchordoquy et al. 2017	Copper
Rat hepatocytes	DNA strand breaks	NT	+	Sina et al. 1983	Copper sulfate
Rat hepatocytes	Unscheduled DNA synthesis	+	+	Denizeau and Marion 1989	Copper sulfate

+ = positive results; – = negative results; NT = not tested

2.21 COPPER NANOPARTICLES

The following section provides a brief overview on toxicity of copper nanoparticles (CuNPs), including copper oxide nanoparticles (CuONPs) when indicated, and is focused on highlighting findings from experimental animal studies. Occupational populations are more likely to be exposed to CuNPs than the general population, and emissions may come from industrial facilities such as for asphalt and rubber production (Ameh and Sayes 2019). CuNPs 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 CuNPs from applied agricultural products, and these plants can present another potential source of human exposure (Ameh and Sayes 2019). No epidemiology studies using CuNPs were identified. *In vitro* models using human cell lines have demonstrated that CuNPs 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 CuNPs in animals are limited but suggest that CuNPs are toxic in laboratory animals. Several *in vivo* and *in vitro* studies have demonstrated that CuNPs 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 CuNP toxicity include the liver, kidneys, and spleen. Oral administration of copper oxide nanoparticles (CuONPs) 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 et al. 2018). Hepatic effects in rats and mice resulting from acute oral exposure to CuONPs or copper carbonate NPs 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 CYP450 enzyme activities (El Bialy et al. 2020; Chen et al. 2006; De Jong et al. 2019; Tang et al. 2018). Oral exposure to CuONPs 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 CuNPs include glomerular hypercellularity, severe coagulative necrosis, detached tubular epithelia, loss of brush border, and narrowing of tubular lumen (El Bialy et al. 2020; Chen et al. 2016; De Jong et al. 2019). In the spleen, CuNPs resulted in splenic, lymphatic and thymus atrophy and lymphoid depletion in rats and mice after acute oral exposure (El Bialy et al. 2020; Chen et al. 2016; De Jong et al. 2019).

Other adverse effects that were observed in animals exposed to CuNPs include evidence for neurological, gastrointestinal, and pulmonary toxicity. Neurotoxic findings following oral or intravenous CuNP injection in rodents include changes in motor activity, 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, CuNPs 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 CuNPs 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 CuNP exposure to carcinogenicity.

Hematological effects in rats and mice from CuNP exposure include decreased red blood cell counts, white blood cell counts, hematocrits, and hemoglobin levels (El Bialy et al. 2020; De Jong et al. 2019). CuNPs 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 CuNPs 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 CuNPs was more toxic to the reproductive functioning of male mice than copper sulfate exposure. CuNPs 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 CuNPs can vary widely depending on particle size, other physicochemical properties, and the preparation. Identified studies were limited to inhalation and ingestion of CuNPs. 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 which transport copper across the membranes as required (Haywood 2019). CuNPs can be distributed throughout the body. The primary target organs in animals tend to be the brain, liver, kidney, and spleen where the CuNPs induce pathological changes and organ injuries. It is hypothesized that the smaller particle size of CuNPs increases surface area which in turn increases its reactivity with hydrogen ions in gastric fluids. This in turn 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 CuNPs are primarily excreted in the feces of mammals with minimal excretion in urine.

Evidence to date suggests that CuNPs and soluble copper compounds share several target organs including the liver, kidney, and stomach. Specifically, since CuNPs 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 CuNP 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).