CHAPTER 2. HEALTH EFFECTS

OVERVIEW

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 nickel. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

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

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

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

Summaries of cardiovascular human epidemiological studies are presented in Table 2-4. Animal inhalation studies are presented in Table 2-1 and Figure 2-2; and human and animal oral studies are presented in Table 2-2 and Figure 2-21; dermal data are presented in Table 2-3.

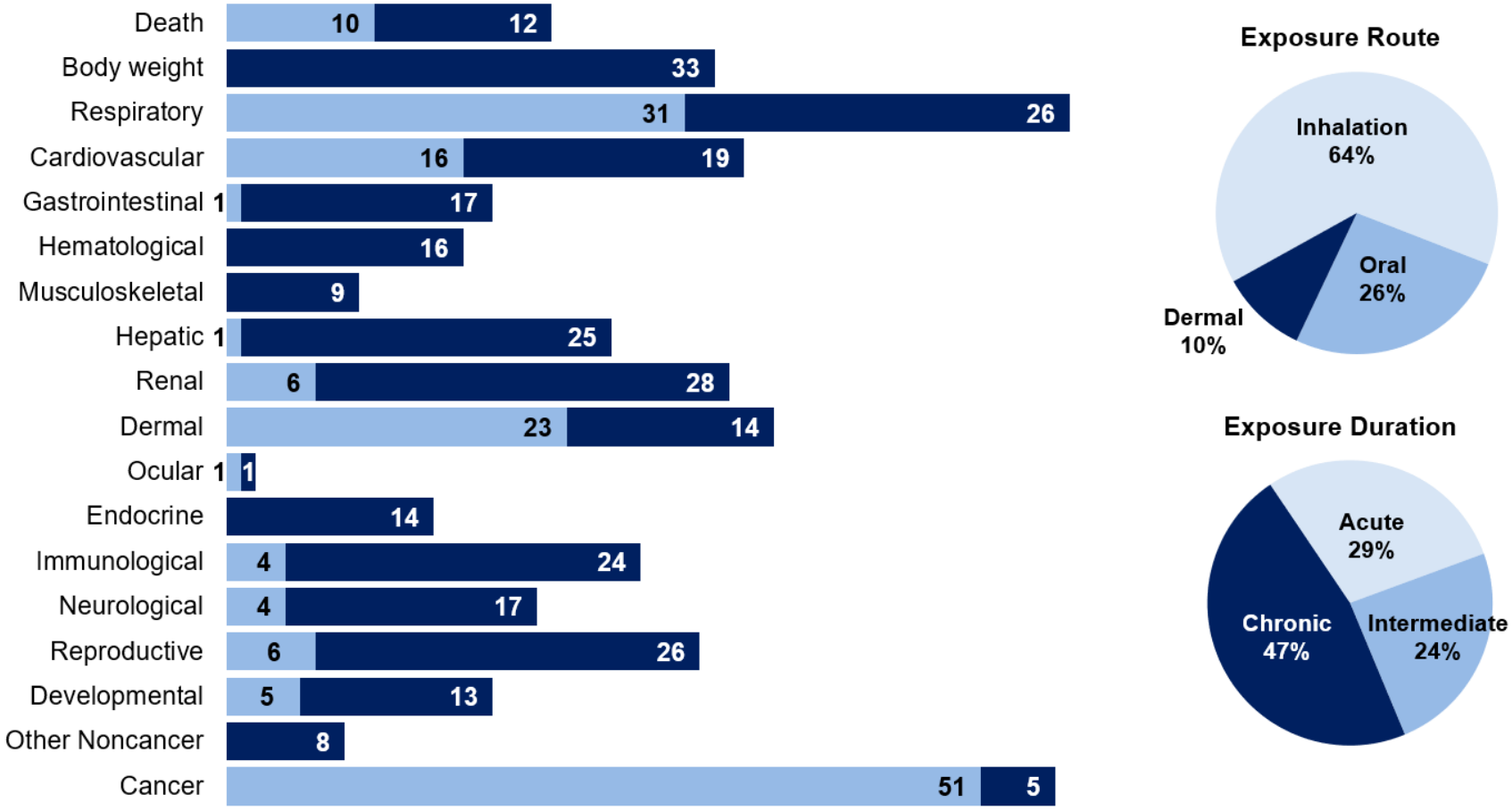
Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the 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 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 Nickel Health Effects*

Most studies examined the potential respiratory and cancerous effects of nickel exposure.
More studies have evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint).



*Includes studies discussed in Chapter 2. A total of 210 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Bai et al. 2013									**Nickel carbonyl**
1	RAT (Sprague-Dawley) 40B	30 minutes (NS)	0, 6.88, 46.47, 85.94	HP	Resp		6.88	85.94	Damage of type II alveolar epithelial cells in rat lung tissue at 6.88 mg Ni/m^3 Pulmonary tissue edema, decreased peroxidation of pulmonary tissue lipid at 85.94 mg Ni/m^3
Benson et al. 1995b									**Nickel subsulfide**
2	RAT (Fischer-344) 4-6B	1, 2, 4, 7, or 12 days 6 hours/day	0, 0.44, 1.83	BC BW HP	Bd wt	0.44	1.83		~17-19% less body weight at day 7 of exposure
					Resp			0.44	Alveolitis in 6/6 rats, type II cell hypertrophy in 1/6 rats among other lung lesions after 7 days of exposure
Efremenko et al. 2014									**Nickel subsulfide**
3	RAT (Fischer-344) 5M	1 week 5 days/week 6 hours/day	0, 0.03, 0.06, 0.11, 0.43	BW BI	Bd wt	0.43			
					Resp	0.11	0.43		Over 250% increase of LDH in BALF
Efremenko et al. 2014									**Nickel subsulfide**
4	RAT (Fischer-344) 5M	1 week 5 days/week 6 hours/day	0, 0.43	BI GN HP	Resp		0.43		Peribronchiolar/perivascular inflammation in 5/5 rats
Hirano et al. 1994									**Nickel sulfate**
5	RAT (Wistar) 28M	2 hours	36.5	LE	Death			36.5	4/28 died
NTP 1996a									**Nickel oxide**

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
6	RAT (Fischer-344) 5M, 5F	12 days in 16-day period 6 hours/day	0, 0.9, 2.0, 3.9, 7.9, 23.6	BW CS HE HP LE OW	Bd wt	23.6			
					Resp	3.9	7.9		Lung inflammation in 2/5 male rats and 5/5 female rats
					Cardio	23.6			
					Gastro	23.6			
					Musc/skel	23.6			
					Hepatic	23.6			
					Renal	23.6			
					Dermal	23.6			
					Endocr	23.6			
					Immuno	23.6			
					Neuro	23.6			
					Repro	23.6			
NTP 1996b									**Nickel subsulfide**
7	RAT (Fischer-344) 5M, 5F	12 days in 16-day period 6 hours/day	0, 0.44, 0.88, 1.83, 3.65, 7.33	BW CS HE HP LE OW	Bd wt	1.83		3.65	22-28% decrease in body weight gain
					Resp		0.44		Chronic lung inflammation (10/10 rats), atrophy of olfactory epithelium (6/10 rats)
								3.65 F	Labored respiration
								7.33 M	Labored respiration
					Cardio	7.33			
					Gastro	7.33			
					Hepatic	7.33			
					Renal	7.33			
					Dermal	7.33			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Endocr	7.33			
					Immuno	7.33			
					Neuro	7.33			
					Repro	7.33			
NTP 1996c									**Nickel sulfate hexahydrate**
8	RAT (Fischer-344) 5M, 5F	12 days in 16-day period 6 hours/day	0, 0.7, 1.4, 3.1, 6.1, 12.2	BW HE HP LE OW	Death			12.2 F	5/5 died
					Bd wt			0.7 M	Final body weights 28% lower than controls
					Resp			0.7	Labored breathing and increased respiration rates; chronic lung inflammation, degeneration of bronchiolar epithelium, and atrophy of olfactory epithelium in 10/10 rats
					Cardio	12.2			
					Gastro	12.2			
					Musc/skel	12.2			
					Hepatic	12.2			
					Renal	12.2			
					Dermal	12.2			
					Endocr	12.2			
					Immuno	0.7 F	1.4 F		Hyperplasia in bronchial (7/9 rats) and mediastinal (5/8 rats) lymph nodes
					Neuro	3.1 F			
					Repro	12.2			
Adkins et al. 1979a									**Nickel chloride**

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
9	MOUSE (CD-1) 113F	2 hours	0, 0.66	BI CS	Immuno			0.66	Decreased ability to clear bacteria from lungs resulting in a significant increase in mortality (>20% higher than controls) and increased incidence of sepsis
Adkins et al. 1979b									**Nickel sulfate**
10	MOUSE (CD-1) 120F	2 hours	0, 0.46	BI CS	Immuno			0.46	Increased susceptibility to Streptococcal infection resulting in a significant increase in mortality (21% higher than controls) and reduced mean survival time (2 days less than controls)
Adkins et al. 1979c									**Nickel chloride**
11	MOUSE (CD-1) 80-160F	2 hours	0, 0.288, 0.292, 0.369, 0.5, 0.51	BI CS	Immuno	0.37		0.5	Increased susceptibility to Streptococcal infection resulting in a significant increase in mortality (26% higher than controls) and reduced mean survival time (2.73 days less than controls)
Buxton et al. 2021									**Nickel chloride hexahydrate**
12	MOUSE (ICR) 10-15F	24 hours	0, 0.02, 0.04, 0.08	BW CS FI GN HP OW WI	Bd wt	0.08			
					Immuno	0.08			
Graham et al. 1978									**Nickel chloride**
13	MOUSE (Swiss) 14-29F	2 hours	0, 0.1, 0.25, 0.35, 0.5	OF OW	Immuno	0.1	0.25		Impaired humoral immunity
NTP 1996a									**Nickel oxide**

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
14	MOUSE (B6C3F1) 5M, 5F	12 days in 16-day period 6 hours/day	0, 0.9, 2.0, 3.9, 7.9, 23.6	BW CS HE HP LE OW	Bd wt	23.6			
					Resp	3.9	7.9		Elevated incidence of alveolar macrophage hyperplasia in 5/10 males and 3/10 females
					Cardio	23.6			
					Gastro	23.6			
					Hepatic	23.6			
					Renal	23.6			
					Dermal	23.6			
					Endocr	23.6			
					Immuno	23.6			
					Neuro	23.6			
					Repro	23.6			
NTP 1996b									**Nickel subsulfide**
15	MOUSE (B6C3F1) 5M, 5F	12 days in 16 day period 6 hours/day	0, 0.44, 0.88, 1.83, 3.65, 7.33	BW HE HP LE OW	Death			7.33	10/10 died
					Bd wt	3.65 F			
						1.83 M	3.65 M		14% less body weight
					Resp	0.44	0.88	7.33	Atrophy of olfactory epithelium in 5/10 mice at 0.88 mg Ni/m^3 Necrosis in alveolar and bronchiolar epithelium, extensive vascular congestion, and edema at 7.33 mg Ni/m^3
					Gastro	3.65			
					Hemato	3.65			
					Musc/skel	3.65			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic	3.65			
					Renal	3.65			
					Dermal	3.65			
					Endocr	3.65			
					Immuno	0.44	0.88		Lymphoid hyperplasia in bronchial lymph nodes in 3/3 males and 1/2 females
					Neuro	3.65			
					Repro	3.65			
NTP 1996c									**Nickel sulfate hexahydrate**
16	MOUSE (B6C3F1) 5M, 5F	12 days in 16 day period 6 hours/day	0, 0.7, 1.4, 3.1, 6.1, 12.2	BW CS HE HP LE OW	Death			1.4	10/10 died
					Bd wt	0.7			
					Resp		0.7	1.4	Chronic lung inflammation in 9/10 mice and olfactory epithelium atrophy in 10/10 mice at 0.7 mg Ni/m^3 Necrotizing inflammatory lesions with edema, vascular congestion in all mice; rapid respiration rates at 1.4 mg Ni/m^3
					Cardio	1.4			
					Gastro	1.4			
					Musc/skel	1.4			
					Hepatic	1.4			
					Renal	1.4			
					Dermal	1.4			
					Endocr	1.4			

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Immuno	3.1			
					Neuro	0.7			
					Repro	1.4			
Muggenburg et al. 2003									**Nickel sulfate**
17	DOG (Beagle) 4B	3 hours	0.05, 0.1	CS	Cardio	0.1			
Muggenburg et al. 2003									**Nickel oxide**
18	DOG (Beagle) 4B	3 hours	0.06	CS	Cardio	0.06			
INTERMEDIATE EXPOSURE									
Benson et al. 1995a									**Nickel oxide**
19	RAT (Fischer-344) 90M	2-6 months 5 days/week 6 hours/day	0, 0.49, 1.96	BW CS HP OW	Bd wt	1.96			
					Resp	0.49	1.96		Moderate alveolitis that persisted at least 4 months after the exposure
Benson et al. 1995a									**Nickel sulfate**
20	RAT (Fischer-344) 90M	2-6 months 5 days/week 6 hours/day	0, 0.03, 0.11	BW CS HP OW	Resp	0.03	0.11		Alveolitis that persisted for 4 months after exposure
Benson et al. 1995b									**Nickel subsulfide**
21	RAT (Fischer-344) 4-6B	22 days 6 hours/day	0, 0.44, 1.83	BI BW HP OW	Bd wt	0.44	1.83		~10-19% less body weight
					Resp			0.44	Alveolitis in 6/6 rats, alveolar proteinosis in 5/6 rats, and olfactory epithelium degeneration in 3/4 rats; 18-27% increase in lung weight

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Bingham et al. 1972									**Nickel oxide**
22	RAT (Wistar) 10M	> 2 weeks 6 days/week 12 hours/day	0, 0.12	BI CS HP	Resp		0.12		Alveolar wall thickening
Bingham et al. 1972									**Nickel chloride**
23	RAT (Wistar) 10M	>2 weeks 6 days/week 12 hours/day	0, 0.109	BI CS HP	Resp		0.11		Hyperplasia of the bronchial epithelium and peribronchial lymphocytic infiltration
Efremenko et al. 2014									**Nickel subsulfide**
24	RAT (Fischer-344) 26M (5M for HP)	4 weeks 5 days/week 6 hours/day	0, 0.03, 0.06, 0.11, 0.45	BW BI CS GN HP	Bd wt	0.45 M			
					Resp	0.06 M	0.11 M		Lung alveolus inflammation in 5/5 rats; significantly increased lymphocytes and macrophages
Evans et al. 1995									**Nickel sulfate**
25	RAT (Long-Evans) 5-14M	16 days 6 hours/day	0, 0.635	BW HP NX OW	Bd wt	0.64			
					Resp		0.64		Atrophy of olfactory epithelium; significant 20% increase in relative lung weight
					Renal	0.64			
					Neuro		0.64		Decrease in number of bipolar receptor cells in nasal olfactory epithelium
Horie et al. 1985									**Nickel oxide**
26	RAT (Wistar) 2-8M	1 month 5 days/week 6 hours/day	0, 0.5, 1.1, 5.1, 5.5, 6.3	CS HP	Resp		0.5		Bronchial gland hyperplasia in 5/6 rats, and squamous metaplasia in 3/6 rats
					Cancer			0.5	Adenocarcinoma in 1/6 rats

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Morimoto et al. 1995									**Nickel oxide**
27	RAT (Wistar) 5M	4 weeks 5 days/week 8 hours/day	0, 9.2	BC	Immuno		9.2		Increased production of tumor necrosis factor by alveolar macrophages
NTP 1996a									**Nickel oxide**
28	RAT (Fischer-344) 10M, 10F	13 weeks 5 days/week 6 hours/day	0, 0.4, 0.9, 2.0, 3.9, 7.9	BW CS HE HP LE OW RX	Bd wt	7.9			
					Resp	2	3.9		Chronic active lung inflammation (17/20 rats), granulomatous inflammation (7/20 rats), and lung interstitial infiltrate in all rats
					Cardio	7.9			
					Gastro	7.9			
					Musc/skel	7.9			
					Hepatic	7.9			
					Renal	7.9			
					Dermal	7.9			
					Endocr	7.9			
					Immuno	2	3.9		Chronic active lung inflammation in 17/20 rats
					Neuro	7.9			
					Repro	7.9 F			
						3.9 M		7.9 M	20.5% decrease in sperm concentration
NTP 1996b									**Nickel subsulfide**
29		13 weeks 5 days/week			Bd wt	1.83			
					Resp	0.11	0.22		Chronic inflammation in 9/10 rats

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
	RAT (Fischer-344) 10M, 10F	6 hours/day	0, 0.11, 0.22, 0.44, 0.88, 1.83	BW CS HE HP LE OW RX				1.83 M	Labored breathing during weeks 2-7
					Cardio	1.83			
					Gastro	1.83			
					Hemato	0.11 F	0.22 F		3% increase in erythrocytes (p<=0.01)
						0.44 M	0.88 M		4 and 4.5% increase of erythrocyte and hemoglobin levels, respectively
					Musc/skel	1.83			
					Hepatic	1.83			
					Renal	1.83			
					Dermal	1.83			
					Endocr	1.83			
					Immuno	0.22	0.44		Lymphoid hyperplasia in bronchial (19/20 rats) and mediastinal (14/19 rats) lymph nodes
					Neuro	1.83			
					Repro	1.83			
NTP 1996c									**Nickel sulfate hexahydrate**
30	RAT (Fischer-344) 10M, 10F	13 weeks 5 days/week 6 hours/day	0, 0.03, 0.06, 0.11, 0.22, 0.44	BW CS HE HP LE OW RX	Bd wt	0.44			
					Resp	0.06 F[b]	0.11 F		Chronic lung inflammation in 4/10 rats and interstitial infiltrates in 6/10 rats ($NOAEL_{HEC,ADJ}$=0.001 mg/m^3)
						0.11 M	0.22 M		Atrophy of olfactory epithelium
					Cardio	0.44			
					Gastro	0.44			
					Musc/skel	0.44			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic	0.44			
					Renal	0.44			
					Dermal	0.44			
					Endocr	0.44			
					Immuno	0.11	0.22		Lymphoid hyperplasia in bronchial (17/19 rats) and mediastinal (17/20 rats) lymph nodes
					Neuro	0.44			
					Repro	0.44			
Oller et al. 2022									**Nickel subsulfide**
31	RAT (Fischer-344) 13M	3 - 13 weeks 5 days/week 6 hours/day	0, 0.04, 0.11, 0.44	BW CS GN HP OW	Bd wt	0.44			
					Resp		0.04		Increased incidence and severity of lung lesions including alveolitis (7/13 rats) and perivascular/ peribronchiolar inflammation (7/13 rats)
Oller et al. 2022									**Nickel sulfate hexahydrate**
32	RAT (Fischer-344) 13M	3 - 13 weeks 5 days/week 6 hours/day	0, 0.03, 0.11, 0.22	BW CS GN HP OW	Bd wt	0.22			
					Resp	0.03	0.11		Increased incidence and severity of lung lesions including alveolitis (7/13 rats) and perivascular/ peribronchiolar inflammation (8/13 rats)
Oller et al. 2022									**Nickel sulfate hexahydrate**
33		3 - 13 weeks 5 days/week	0, 0.44	BW CS GN HP OW	Death			0.44	12 of 13 rats died within 1 week of exposure

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
	RAT (Fischer-344) 13M	6 hours/day			Resp			0.44	Severe pulmonary edema, labored breathing
Spiegelberg et al. 1984									**Nickel oxide**
34	RAT (Wistar) 12M	4 weeks continuous	0, 0.047, 0.093, 0.216, 0.404, 0.818	CS OF	Immuno	0.093	0.216		Impaired humoral immunity
Spiegelberg et al. 1984									**Nickel oxide**
35	RAT (Wistar) 12M	4 months continuous	0, 0.025, 0.145	CS OF	Immuno		0.025		Increased number of macrophages and phagocytic activity increase to 130%
Weischer et al. 1980									**Nickel oxide**
36	RAT (Wistar) 10M	28 days 23.6 hours/day	0, 0.178, 0.385, 0.784	BC BW OW	Bd wt	0.178		0.385	30% decrease in body weight gain
					Resp			0.178	Increased lung weight (31%)
					Hemato		0.178		4% decrease in hematocrit levels
					Hepatic	0.784			
					Renal		0.178		16% decrease in urea
					Other noncancer	0.178	0.385		Increased serum glucose (13%)
Weischer et al. 1980									**Nickel oxide**
37	RAT (Wistar) 10-13F	21 days 23.6 hours/day	0, 0.8, 1.6, 3.2	BC BW DX OW	Bd wt			0.8	36% decrease in body weight gain
					Resp			0.8	Increased lung weight (40%)
					Hemato		0.8		Increased hematocrit (9%) and hemoglobin (10%)
					Hepatic	3.2			
					Renal		0.8		Increased urea (94%)

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Other noncancer		0.8		Decreased serum glucose level (19%)
Weischer et al. 1980									**Nickel oxide**
38	RAT (Wistar) 10-13F	GD 1-21 23.6 hours/day	0, 0.8, 1.6, 3.2	BC BW DX OW RX	Develop	0.8	1.6		Decreased fetal body weights (9%)
Benson et al. 1995a									**Nickel oxide**
39	MOUSE (B6C3F1) 108M	2-6 months 5 days/week 6 hours/day	0, 0.98, 3.9	BW CS HP OW	Bd wt	3.9			
					Resp		0.98		Interstitial pneumonia
Benson et al. 1995a									**Nickel sulfate**
40	MOUSE (B6C3F1) 108M	2-6 months 5 days/week 6 hours/day	0, 0.06, 0.22	BW CS HP OW	Resp	0.06	0.22		Interstitial pneumonia
Haley et al. 1990									**Nickel oxide**
41	MOUSE (B6C3F1) 40F	65 days 5 days/week 6 hours/day	0, 0.47, 2.0, 7.9	BI CS	Immuno		0.47		Decreased alveolar macrophage activity
Haley et al. 1990									**Nickel sulfate hexahydrate**
42	MOUSE (B6C3F1) 40F	65 days 5 days/week 6 hours/day	0, 0.027, 0.11, 0.45	CS OF OW	Immuno	0.11	0.45		Decreased resistance to tumor challenge
Haley et al. 1990									**Nickel subsulfide**

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
43	MOUSE (B6C3F1) 40F	65 days 5 days/week 6 hours/day	0, 0.11, 0.45, 1.8	OF OW	Immuno	0.11	0.45		Pulmonary alveolar macrophage phagocytic activity decreased by approximately 66%
NTP 1996a									**Nickel oxide**
44	MOUSE (B6C3F1) 10M, 10F	13 weeks 5 days/week 6 hours/day	0, 0.4, 0.9, 2.0, 3.9, 7.9	BW HE HP LE OW RX	Bd wt	7.9			
					Resp	2 F	3.9 F		Perivascular lymphocytic infiltrates in 6/10 females
						3.9 M	7.9 M		Perivascular lymphocytic infiltrates in 8/10 males
					Cardio	7.9			
					Gastro	7.9			
					Musc/skel	7.9			
					Hepatic	7.9			
					Renal	7.9			
					Dermal	7.9			
					Endocr	7.9			
					Immuno	3.9	7.9		Increased incidence of bronchial lymph node hyperplasia (5/9 males, 7/9 females)
					Neuro	7.9			
					Repro	7.9			
NTP 1996b									**Nickel subsulfide**
45	MOUSE (B6C3F1) 10M, 10F	13 weeks 5 days/week 6 hours/day	0, 0.11, 0.22, 0.44, 0.88, 1.83	BW CS HE HP LE OW RX	Bd wt	1.83			
					Resp	0.22	0.44		Atrophy of olfactory epithelium
					Cardio	1.83			
					Gastro	1.83			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hemato	1.83			
					Musc/skel	1.83			
					Renal	1.83			
					Dermal	1.83			
					Endocr	1.83			
					Immuno	0.44 F	0.88 F		Lymphoid hyperplasia in bronchial lymph nodes of 5/7 mice
						0.88 M	1.83 M		Lymphoid hyperplasia in bronchial lymph nodes of 8/8 mice
					Neuro	1.83 F			
					Repro	1.83			
NTP 1996c									**Nickel sulfate hexahydrate**
46	MOUSE (B6C3F1) 10M, 10F	13 weeks 5 days/week 6 hours/day	0, 0.03, 0.06, 0.11, 0.22, 0.44	BW CS HE HP LE OW RX	Bd wt	0.44			
					Resp	0.22 F		0.44 F	Chronic lung inflammation (9/10 females), fibrosis (all males and 8/10 females), and interstitial infiltrate (8/10 males; 8/10 females)
					Cardio	0.44			
					Gastro	0.44			
					Musc/skel	0.44			
					Hepatic	0.44			
					Renal	0.44			
					Dermal	0.44			
					Endocr	0.44			
					Immuno	0.22	0.44		Hyperplasia of bronchial lymph nodes in 8/10 females and 5/8 males

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Neuro	0.44 F			
					Repro	0.44			
Xu et al. 2012									**Nickel sulfate**
47	MOUSE (ApoE-/-) 5-6M	3 months 5 days/week 6 hours/day (Environ)	0, 0.00017	BC BI OW	Bd wt	0.00017			
					Cardio		0.00017		Induced microcirculatory dysfunction indicated by increases in adherent and rolling monocytes in the microcirculation
					Hemato	0.00017			
					Endocr	0.00017			
					Immuno		0.00017		Increased macrophages in lung and eWAT tissues
Ying et al. 2013									**Nickel metallic**
48	MOUSE (ApoE-/-) 6M	14 weeks 6 hours/day, 5 days/week (Environ)	0, 0.0004	BI OF	Cardio		0.0004		Vascular endothelial dysfunction indicated by increased aortic relaxation response to acetylcholine
Johansson and Camner 1986									**Nickel metallic**
49	RABBIT (NS) NR M	1-8 months 5 days/week 6 hours/day	0.2, 1	HP	Resp		0.2		Increased volume density of alveolar type II cells
					Immuno		0.2		Increased number of alveolar macrophages
Johansson and Camner 1986									**Nickel metallic**
50	RABBIT (NS) NR M	1-8 months 5 days/week 6 hours/day	0.3	HP	Resp		0.3		Increased volume density of alveolar type II cells
					Immuno		0.3		Increased number of alveolar macrophages

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Johansson et al. 1980									**Nickel metallic**
51	RABBIT (NS) 6M	3 or 6 months 5 days/week 6 hours/day	0, 1.0	HP	Immuno		1		Inactive macrophage surfaces
Johansson et al. 1987									**Nickel chloride**
52	RABBIT (NS) 8M	4-6 weeks 5 days/week 6 hours/day	0, 0.6	HP CS	Immuno		0.6		Decreased lysozyme activity in alveolar macrophages
Johansson et al. 1988a, 1989									**Nickel chloride**
53	RABBIT (NS) 8M	4 months 5 days/week 6 hours/day	0, 0.6	GN HP	Immuno		0.6		Decreased macrophage lysosomal activity
CHRONIC EXPOSURE									
Hueper 1958									**Nickel metallic**
54	RAT (Wistar) 50M 50F	21 months 4-5 days/week 6 hours/day	15.0	CS LE	Death			15	100/100 died
Hueper 1958									**Nickel metallic**
55	RAT (Bethesda Black) 60F	21 months 4-5 days/week 6 hours/day	15.0	CS LE	Death			15	60/60 died
NTP 1996a									**Nickel oxide**
56	RAT	2 years	0, 0.5, 1, 2		Bd wt	2			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
	(Fischer-344) 65M, 65F	5 days/week 6 hours/day		BW CS HE HP LE OW	Resp		0.5		Chronic lung inflammation and lung alveolus pigmentation in 105/106 rats
					Cardio	2			
					Gastro	2			
					Hemato	2			
					Musc/skel	2			
					Hepatic	2			
					Renal	2			
					Dermal	2			
					Endocr	1 F	2 F		Benign pheochromocytoma (adjusted rate=57%) and adrenal medulla hyperplasia in 22/53 rats
						2 M			
					Immuno		0.5		Lymphoid hyperplasia (7/71 males) and pigmentation (88/101 males and females) in bronchial lymph nodes
					Neuro	2			
					Repro	2			
					Cancer			1	CEL: Increased incidence of alveolar/bronchiolar adenoma or carcinoma
NTP 1996b									**Nickel subsulfide**
57	RAT (Fischer-344) 63M, 63F	2 years 6 hours/day 5 days/week	0, 0.11, 0.73	BW CS HE HP LE OW	Bd wt	0.11	0.73		11-12% decrease in body weight gain
					Resp			0.11	Rapid shallow breathing, chronic inflammation of lung in 104/106 rats and lung fibrosis in 98/106 rats

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Cardio	0.73			
					Gastro	0.73			
					Hemato	0.11	0.73		Increased hematocrit (6-9%), hemoglobin (5-10%) in both sexes and 7% increase of erythrocyte in males
					Musc/skel	0.73			
					Renal	0.73			
					Endocr		0.11		Increased incidence of benign pheochromocytoma in males (adjusted rate=85%)
					Immuno		0.11		Lymphoid hyperplasia in bronchial lymph nodes (25/106 rats)
					Neuro	0.73			
					Repro	0.73			
					Cancer			0.73	CEL: increased incidence of alveolar/bronchiolar adenoma or carcinoma
NTP 1996c									**Nickel sulfate hexahydrate**
58	RAT (Fischer-344) 65M, 65F	2 years 5 days/week 6 hours/day	0, 0.03, 0.06, 0.11	BW CS HE HP LE OW	Bd wt	0.11			
					Resp	0.03[c]		0.06	Chronic inflammation (91/106 rats), fibrosis (80/106), and alveolar proteinosis (34/106) in lung; ($NOAEL_{HEC,ADJ}$=0.00036 mg Ni/m^3)
					Cardio	0.11			
					Gastro	0.11			
					Hemato	0.11			
					Hepatic	0.11			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Renal	0.11			
					Dermal	0.11			
					Endocr	0.11			
					Immuno	0.06	0.11		Lymphoid hyperplasia in bronchial lymph nodes (21/101 rats)
					Neuro	0.11			
					Repro	0.11			
Oller et al. 2008									**Nickel metallic**
59	RAT (Wistar) 50M, 50F	104 weeks 5 days/week 6 hours/day (Environ)	0, 0.1, 0.4, 1.0	BW CS FI GN HE HP LE OW	Death			0.4	Reduced survival by week 103, 72% survival in males and 48% survival in females
					Resp			0.1	Labored breathing; alveolar proteinosis, histiocytosis, and chronic lung inflammation
					Hemato		0.1 F		Moderate hypercellularity of the sternum and femoral bone marrows
							0.1 M		7.5 and 8.3% increase in hemoglobin and hematocrit levels, respectively, at week 78
					Renal		0.1 F		Increased incidence of granular brown pigment in kidneys
						0.1 M	0.4 M		Increased incidence of granular brown pigment in kidneys
					Dermal		0.1		Dermal atonia (decrease in normal skin elasticity)
					Immuno		0.1		Minimal-to-severe histiocyte infiltrate in bronchial lymph node and increased incidence of

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
									extramedullary hematopoiesis in the spleen
					Other noncancer	0.1	0.4		Lower mean food consumption reduced in males from week 58-end of study and in females from weeks 66 to 87
					Cancer			0.4	CEL: Increased incidence of malignant pheochromocytoma in males (5/50) and adrenal cortex carcinoma in females (3/54)
Ottolenghi et al. 1974									**Nickel sulfide**
60	RAT (Fischer-344) 22-39M, 24-32F	78-80 weeks 5 days/week 6 hours/day	0, 0.63	BW CS GN HP	Death			0.63	Less than 5% of rats survived
					Bd wt			0.63	Body weight 20-30% less than controls
					Resp			0.63	Pneumonitis, bronchitis, emphysema; lung hyperplasia in 133/208 rats
					Cardio	0.63			
					Gastro	0.63			
					Hepatic	0.63			
					Renal	0.63			
					Endocr	0.63			
					Immuno	0.63			
					Neuro	0.63			
					Cancer			0.63	CEL: Lung adenomas (15/208 rats), adenocarcinomas (10/208), squamous cell carcinoma (3/208)
Takenaka et al. 1985									**Nickel oxide**

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
61	RAT (Wistar) 20-40M	31 months 7 days/week 23 hours/day	0, 0.06, 0.2	BW CS GN HP	Death			0.06	Decreased mean survival time (88 weeks; 125 weeks for controls)
					Bd wt	0.06			
					Resp			0.06	Six-fold increase in lung weight, congestion, and alveolar proteinosis
Tanaka et al. 1988									**Nickel oxide**
62	RAT (Wistar) 1-5M	3, 6, or 12 months 5 days/week 7 hours/day	0, 0.235, 0.942	BW HP OW	Bd wt	0.942			
					Resp			0.235	Increased incidence of pneumonia and 21% increase in lung weight
					Hepatic	0.942			
					Renal	0.942			
Hueper 1958									**Nickel metallic**
63	MOUSE (C57) 20F	21 months 4-5 days/week 6 hours/day	15.0	CS LE	Death			15	20/20 died
NTP 1996a									**Nickel oxide**
64	MOUSE (B6C3F1) 79M, 76F	2 years 5 days/week 6 hours/day	0, 1.0, 2.0, 3.9	BW CS HE HP LE OW	Bd wt	3.9			
					Resp		1		Chronic lung inflammation (64/133 mice), bronchiolization (59/133), and alveolar proteinosis (20/133)
					Cardio	3.9			
					Gastro	3.9			
					Hemato	3.9			
					Musc/skel	3.9			
					Hepatic	3.9			
					Renal	3.9			
					Dermal	3.9			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Endocr	3.9			
					Immuno		1		Bronchial lymph node hyperplasia
					Neuro	3.9			
					Repro	3.9			
					Cancer			2 F	CEL: increased incidence of alveolar/bronchiolar adenoma (10 mice)
NTP 1996b									**Nickel subsulfide**
65	MOUSE (B6C3F1) 80M, 80F	2 years 6 hours/day 5 days/week	0, 0.44, 0.88	BW CS HE HP LE OW	Bd wt	0.88			
					Resp		0.44		Chronic active lung inflammation (98/118 mice), bronchiolization (106/118), alveolar proteinosis (111/118), and fibrosis in 7/59 females; olfactory epithelium atrophy (38/118 mice)
					Cardio	0.88			
					Gastro	0.88			
					Hemato	0.44 F 0.88 M	0.88 F		6.5% increase of hematocrit
					Hepatic	0.88			
					Renal	0.88			
					Dermal	0.88			
					Endocr	0.88			
					Immuno		0.44		Lymphoid hyperplasia (86/110 mice) and macrophage hyperplasia (91/110) in bronchial lymph nodes
					Neuro	0.88			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Repro	0.88			
NTP 1996c									**Nickel sulfate hexahydrate**
66	MOUSE (B6C3F1) 80M, 80F	2 years 5 days/week 6 hours/day	0, 0.06, 0.11, 0.22	BW HE HP LE OW	Bd wt	0.11 F 0.22 M	0.22 F		12% decreased body weight
					Resp		0.06 F		Chronic active lung inflammation (7/60 rats) and bronchiolization (9/60)
						0.06 M	0.11 M		Chronic active lung inflammation (8/62 rats), bronchiolization (19/62), and olfactory epithelium atrophy (12/61)
					Cardio	0.22			
					Gastro	0.22			
					Hemato	0.22			
					Hepatic	0.22			
					Renal	0.22			
					Dermal	0.22			
					Endocr	0.22			
					Immuno	0.06	0.11		Bronchial lymph node macrophage hyperplasia (22/103 rats)
					Neuro	0.22			
					Repro	0.22			
Hueper 1958									**Nickel metallic**
67	GN PIG (strain 13) 32M, 10F	21 months 4-5 days/week 6 hours/day	15.0	CS LE	Death			15	42/42 died

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

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

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

[b]Used to derive a provisional intermediate-duration inhalation minimal risk level of 0.00003 mg/m^3; the $NOAEL_{HEC,ADJ}$ of 0.001 mg/m^3 was divided by an uncertainty factor of 30 (3 for interspecies extrapolation with dosimetric adjustment and 10 for human variability).
[c]Used to derive a provisional chronic-duration inhalation minimal risk level of 0.00001 mg/m^3; the $NOAEL_{HEC,ADJ}$ of 0.00036 mg/m^3 was divided by an uncertainty factor of 30 (3 for interspecies extrapolation with dosimetric adjustment and 10 for human variability).

B = both sexes; BALF = bronchoalveolar lavage fluid; Bd wt and BW= body weight; BC = serum (blood) chemistry; BI =biochemical changes; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; DX = developmental toxicity; Endocr = endocrine; Environ = environmental; eWAT = epididymal white adipose tissue; F= female(s); FI = food intake; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; HE = hematological; HEC = human equivalent concentration; HP = histopathology; Immuno = immunological; LDH = lactate dehydrogenase; LE = lethality; LC50 = concentration producing 50% death; LOAEL = lowest-observed-adverse-effect-level; M = male(s); Musc/skel = musculoskeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect-level; NR = not reported; NS = not specified; NX = neurological function; OF = organ function; OW = organ weight; Repro = Reproductive; Resp = respiratory; RX = reproductive function; SLOAEL = serious lowest-observed-adverse-effect-level

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

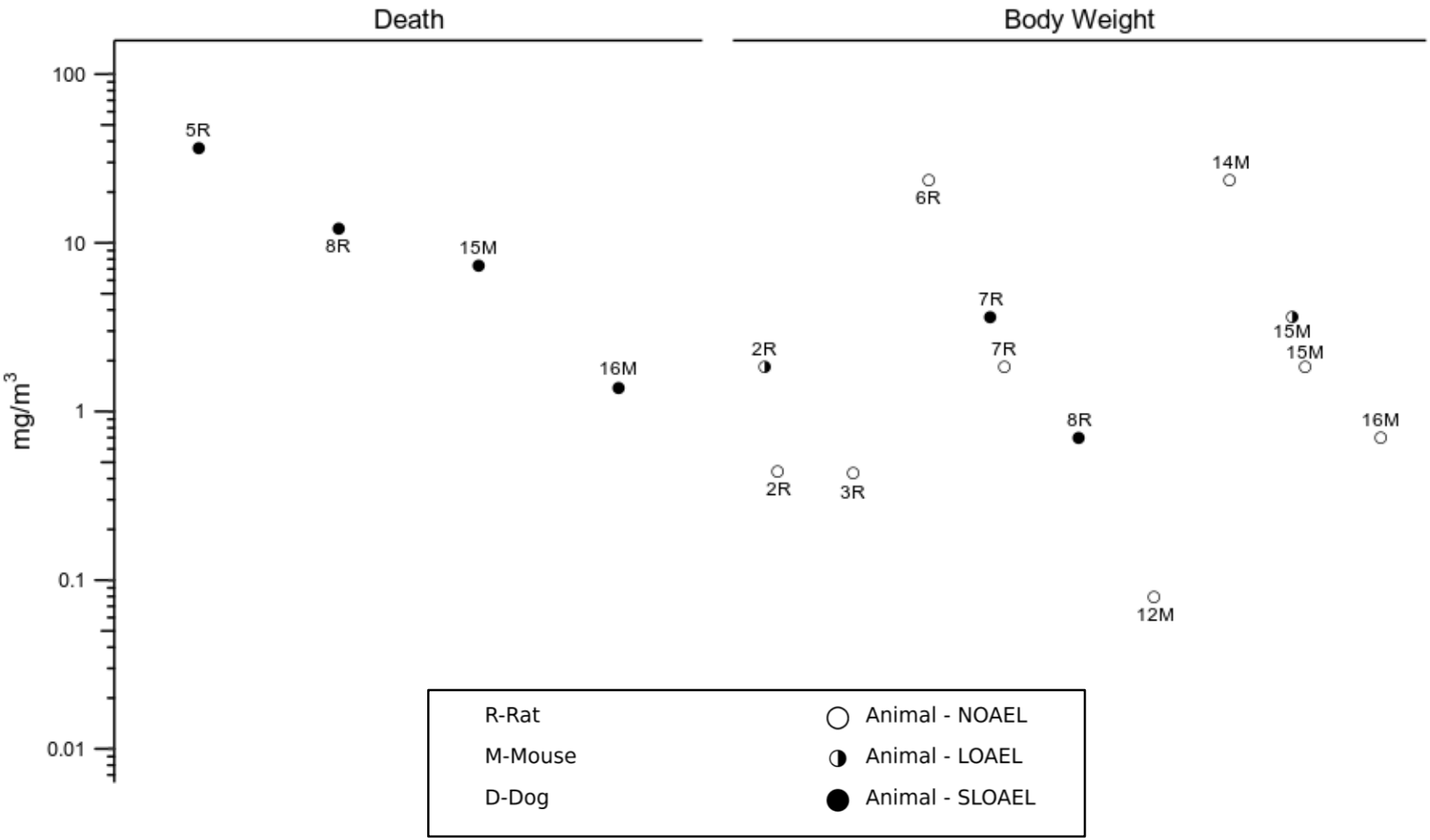

Figure 2-3. Levels of Significant Exposure to Nickel – Inhalation
Acute (≤14 days)

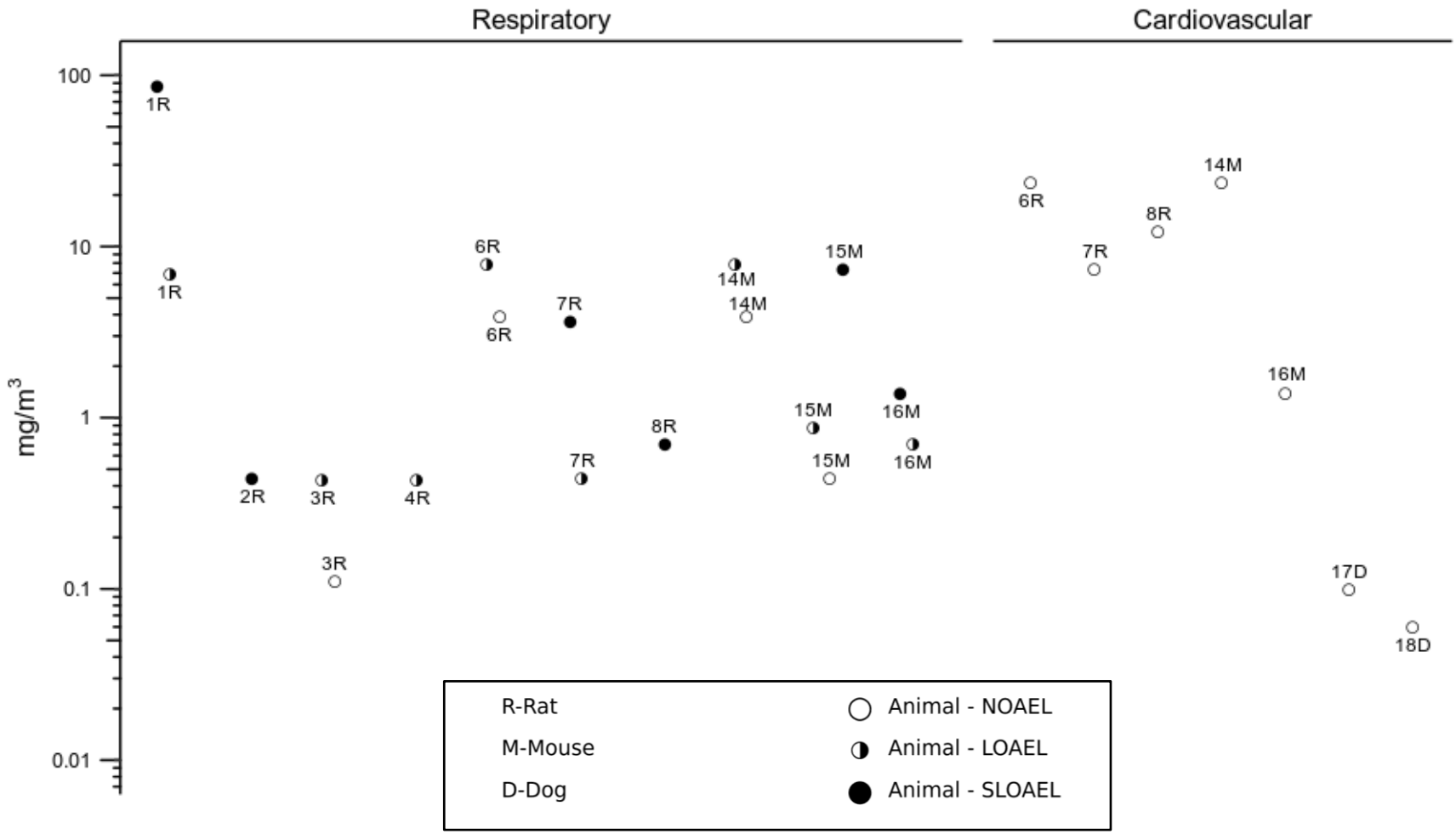

Figure 2-4. Levels of Significant Exposure to Nickel – Inhalation
Acute (≤14 days)

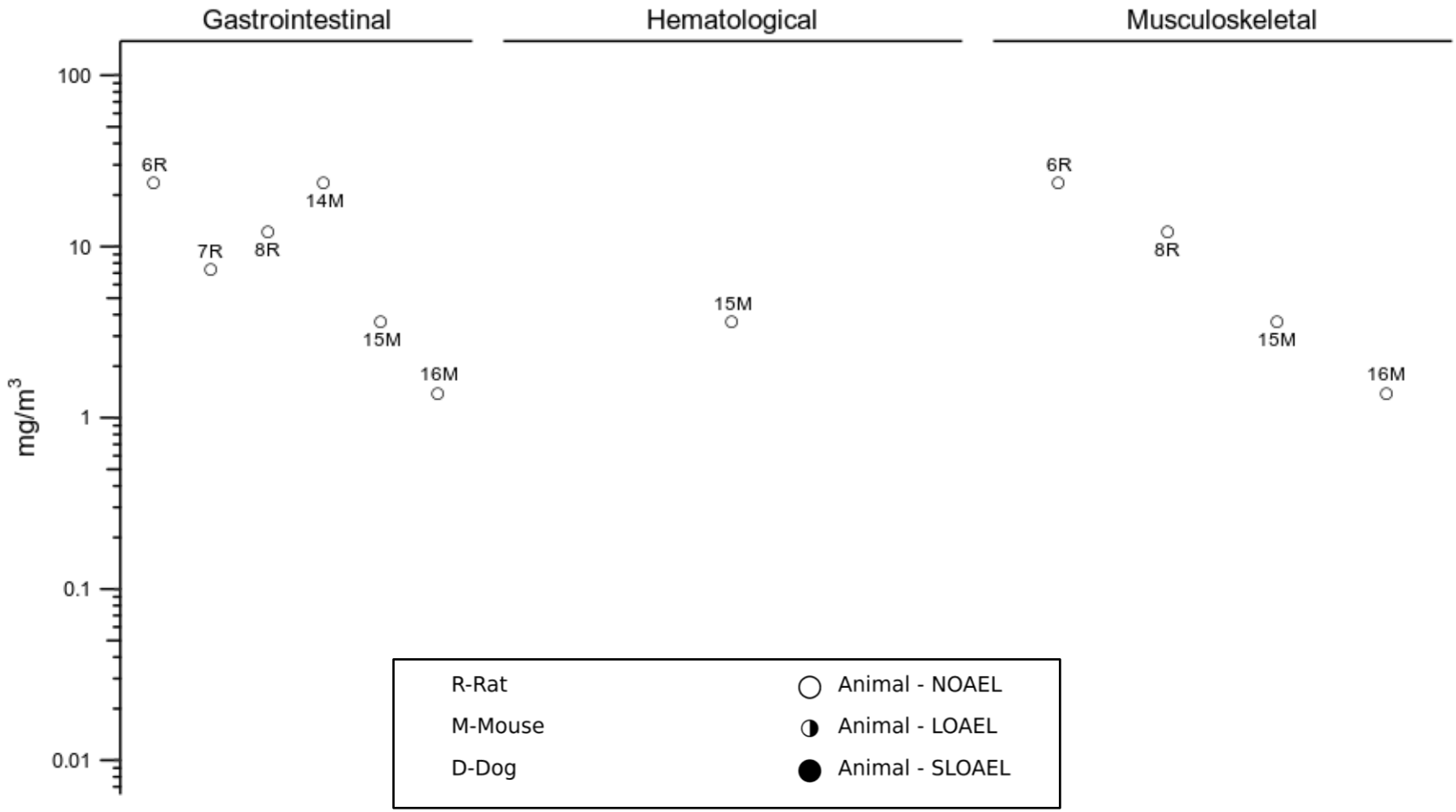

Figure 2-5. Levels of Significant Exposure to Nickel – Inhalation
Acute (≤14 days)

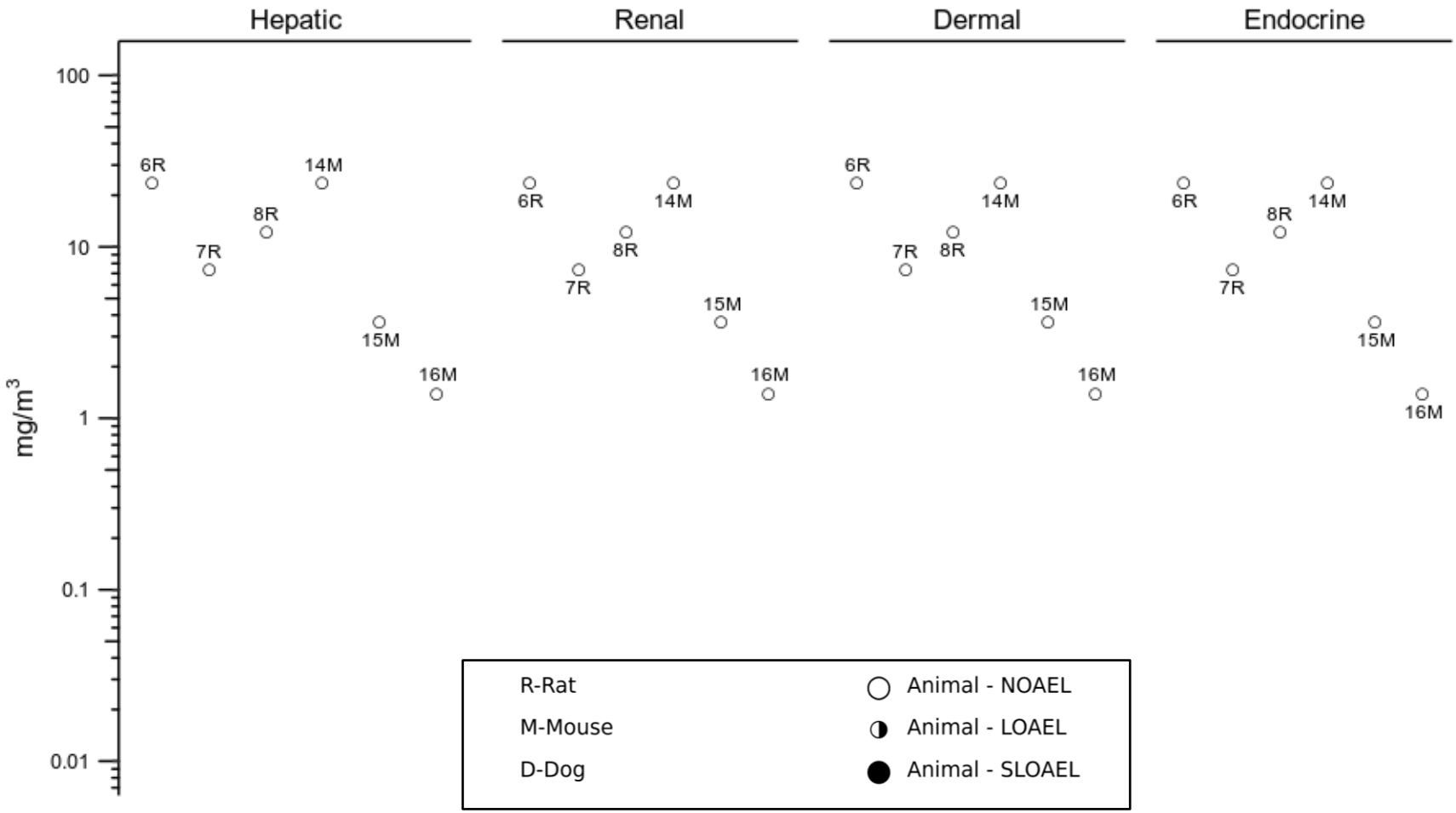

Figure 2-6. Levels of Significant Exposure to Nickel – Inhalation
Acute (≤14 days)

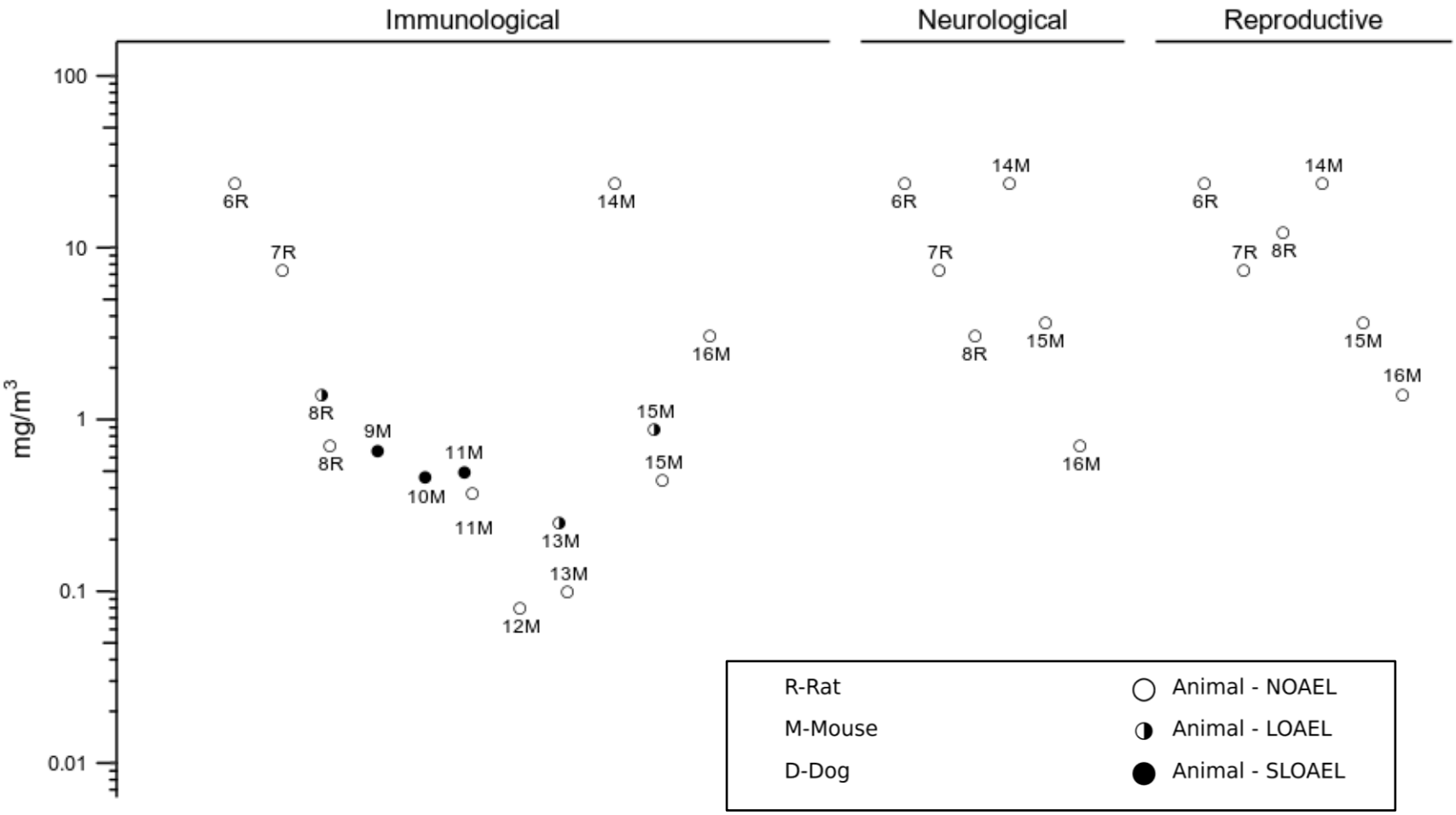

Figure 2-7. Levels of Significant Exposure to Nickel – Inhalation
Intermediate (15-364 days)

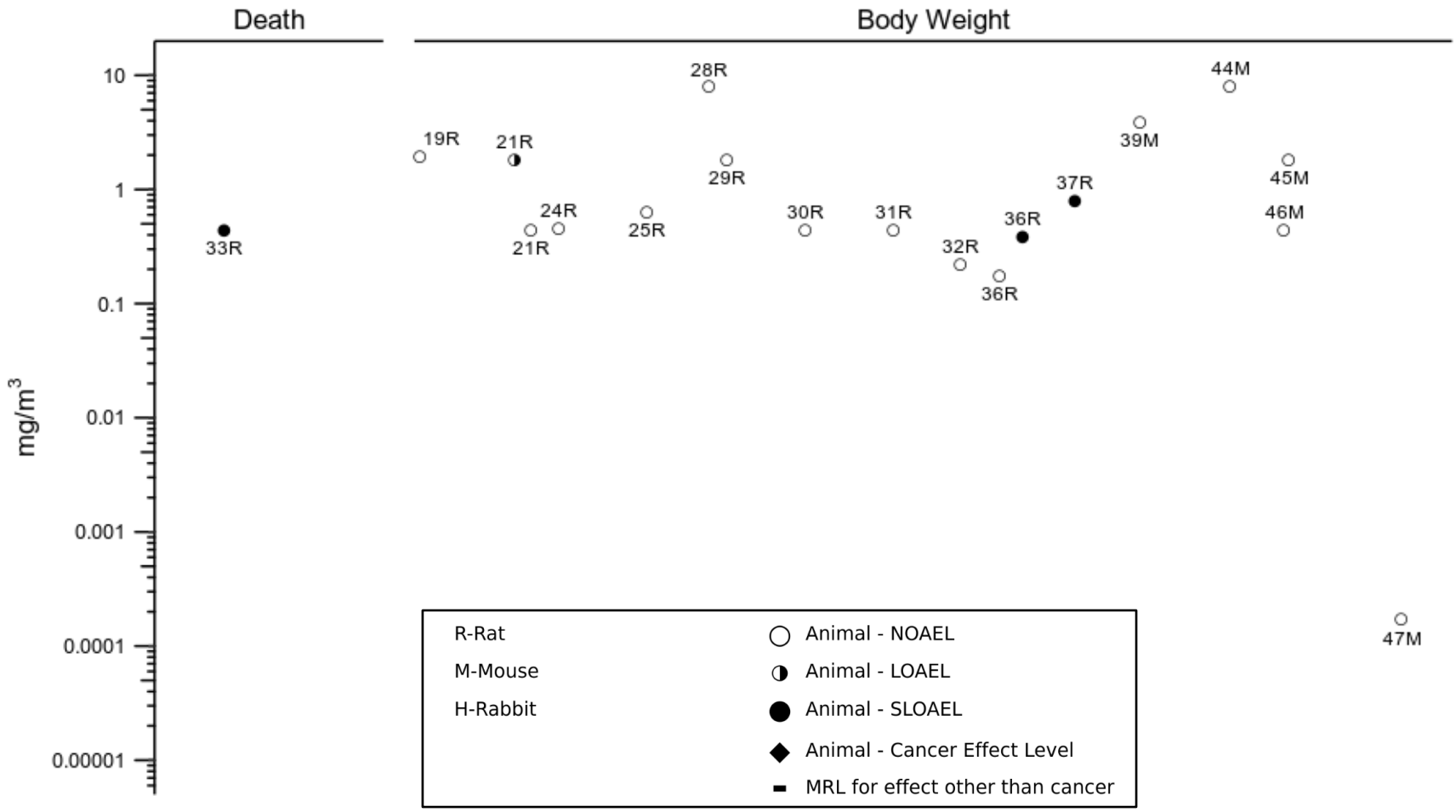

Figure 2-8. Levels of Significant Exposure to Nickel – Inhalation
Intermediate (15-364 days)

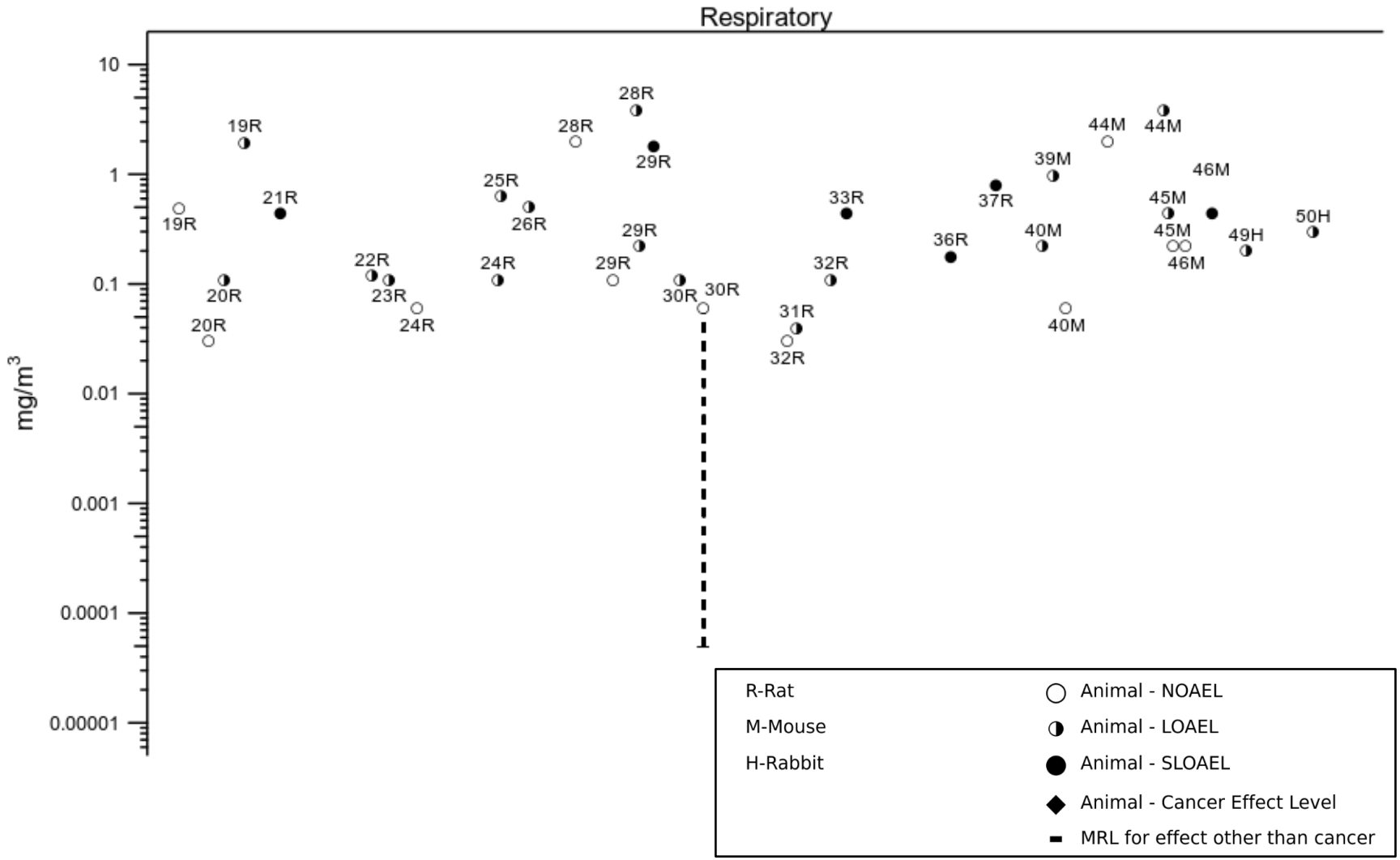

Figure 2-9. Levels of Significant Exposure to Nickel – Inhalation
Intermediate (15-364 days)

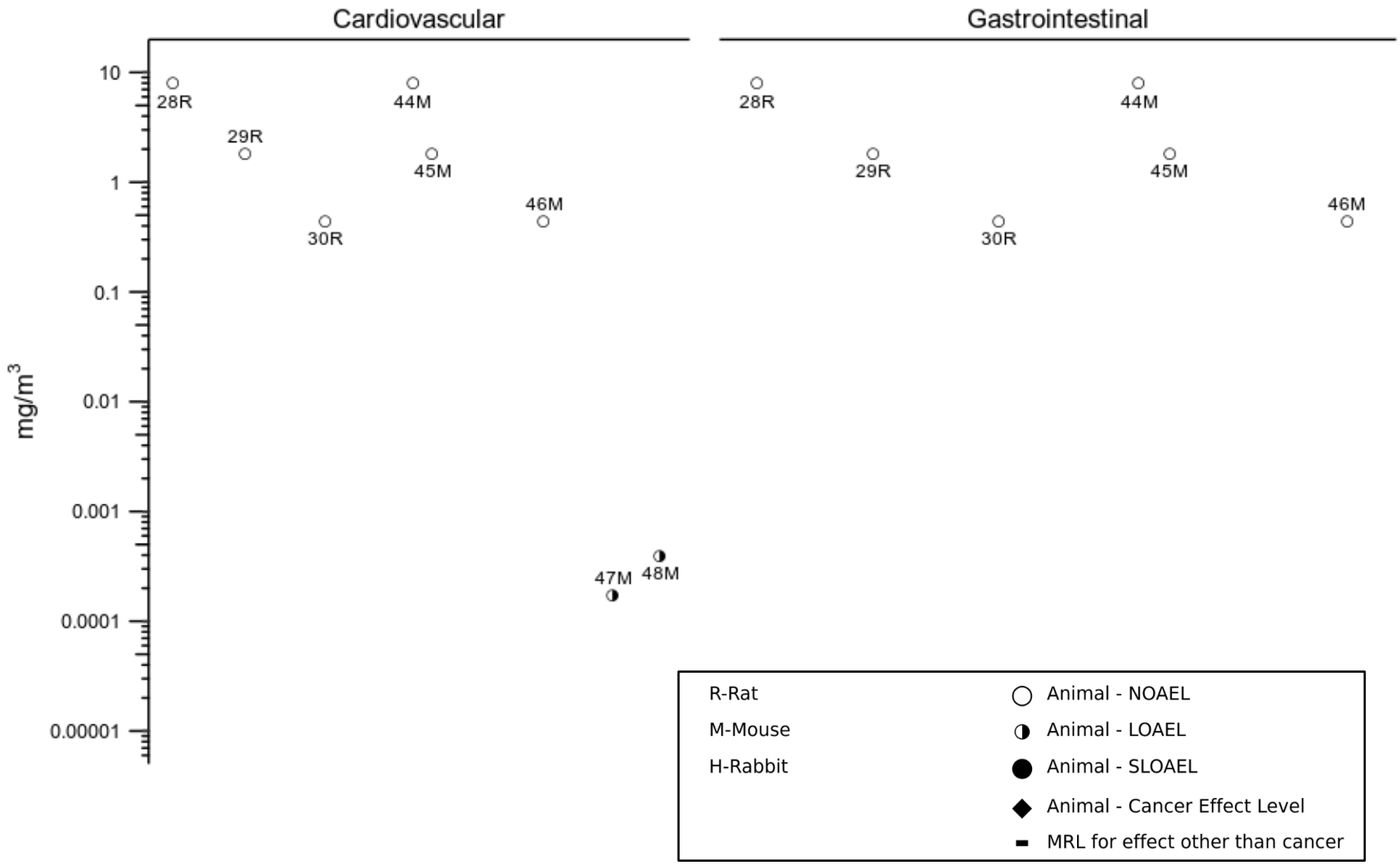

Figure 2-10. Levels of Significant Exposure to Nickel – Inhalation
Intermediate (15-364 days)

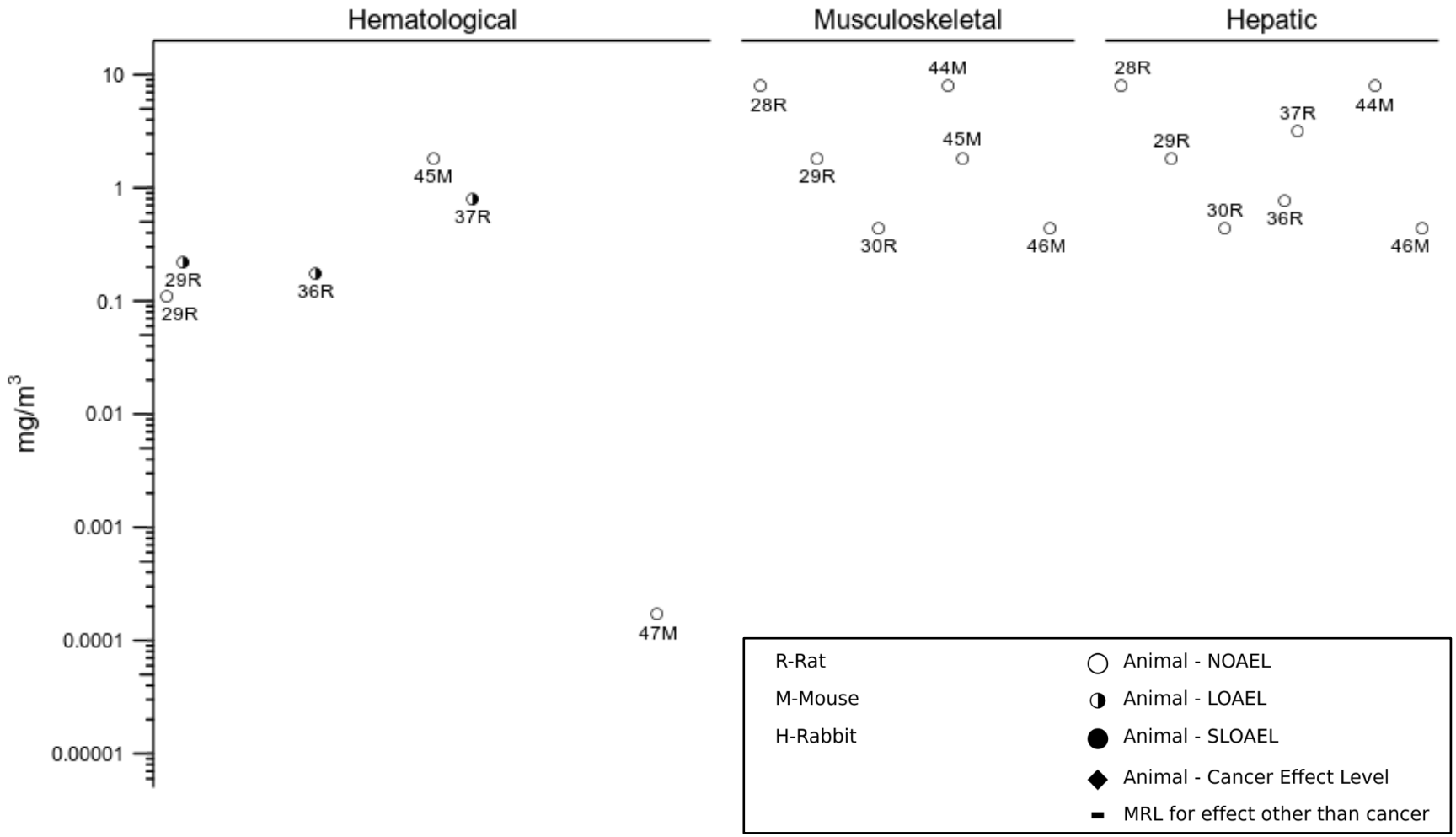

Figure 2-11. Levels of Significant Exposure to Nickel – Inhalation
Intermediate (15-364 days)

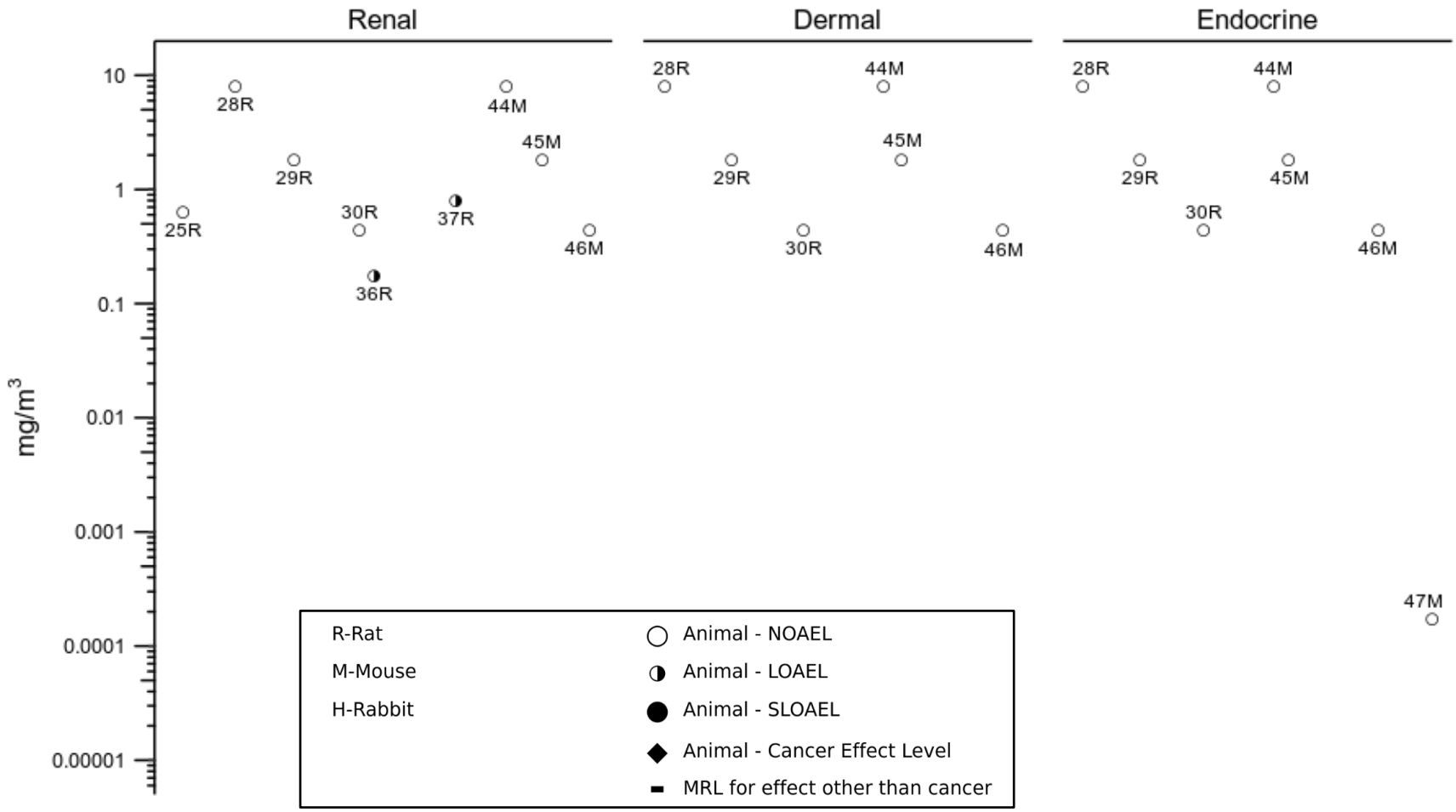

Figure 2-12. Levels of Significant Exposure to Nickel – Inhalation
Intermediate (15-364 days)

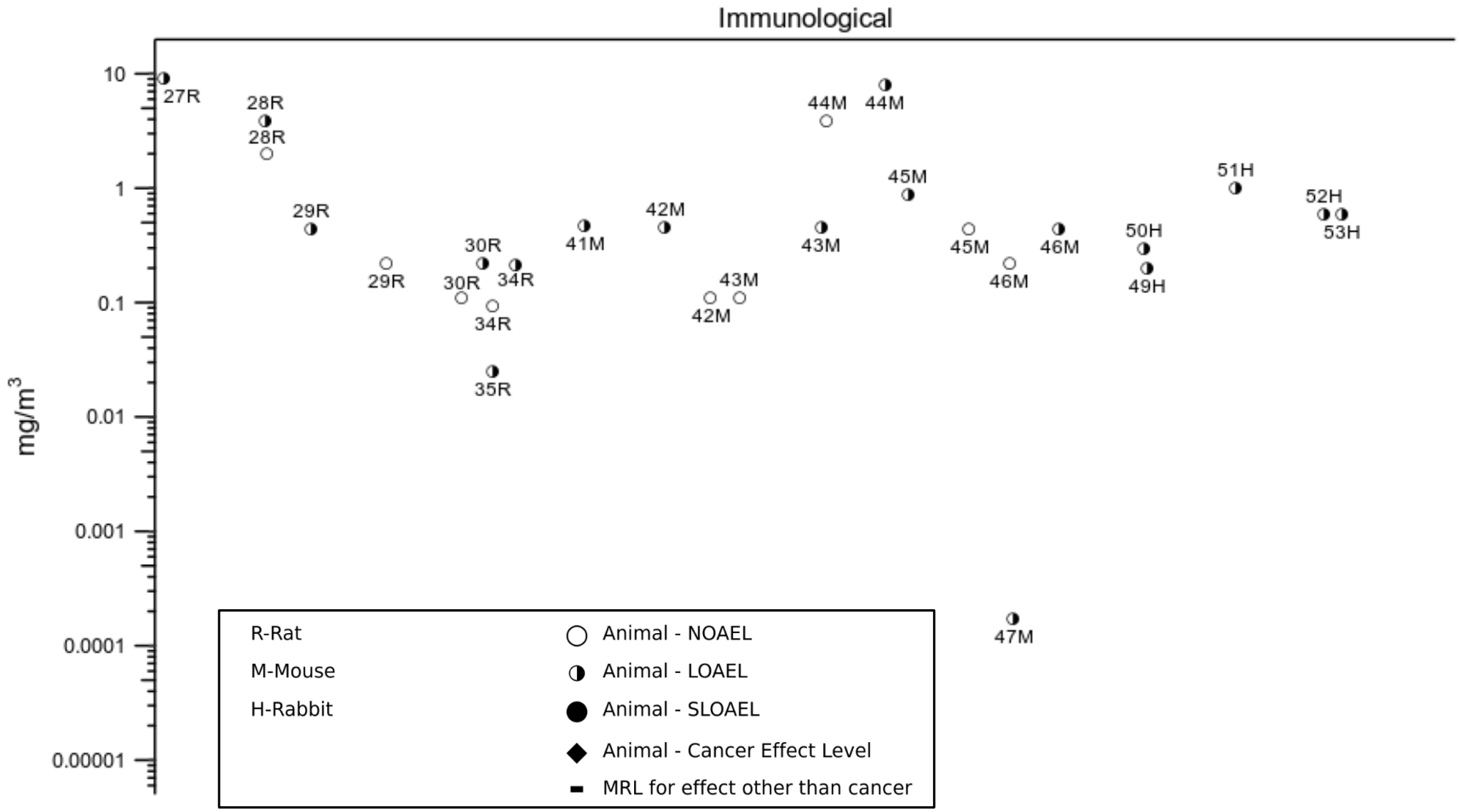

Figure 2-13. Levels of Significant Exposure to Nickel – Inhalation
Intermediate (15-364 days)

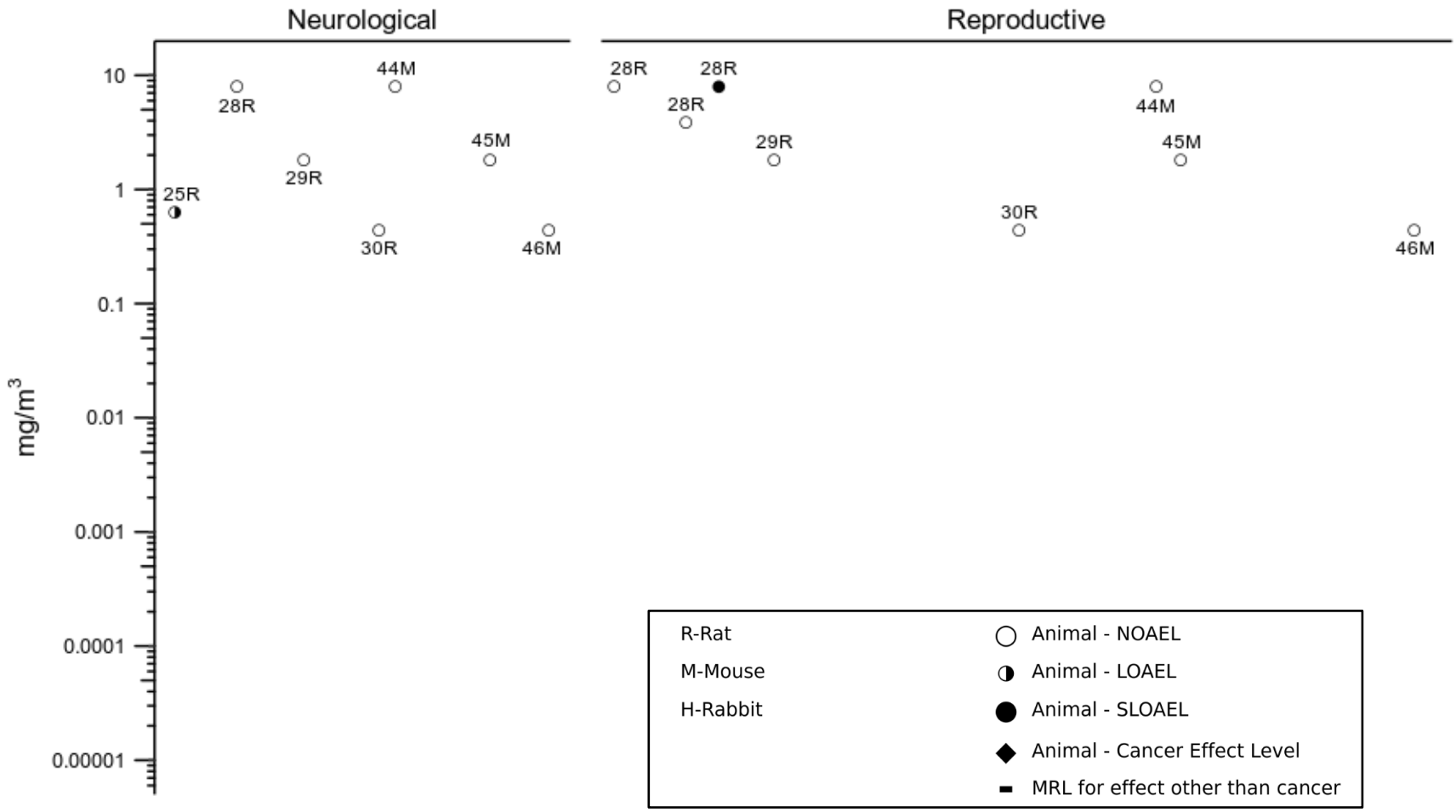

Figure 2-14. Levels of Significant Exposure to Nickel – Inhalation
Intermediate (15-364 days)

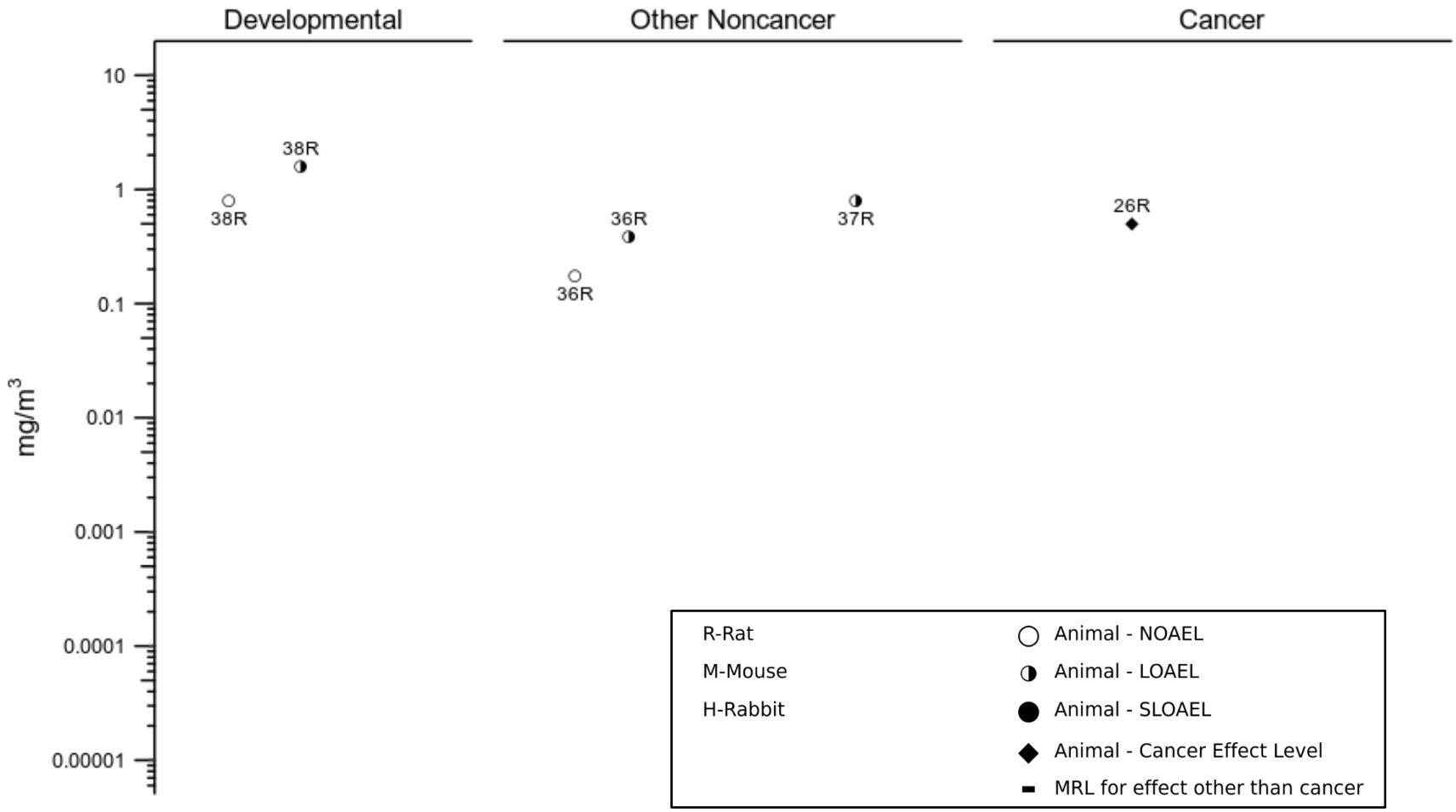

Figure 2-15. Levels of Significant Exposure to Nickel – Inhalation
Chronic (≥365 days)

Figure 2-16. Levels of Significant Exposure to Nickel – Inhalation
Chronic (≥365 days)

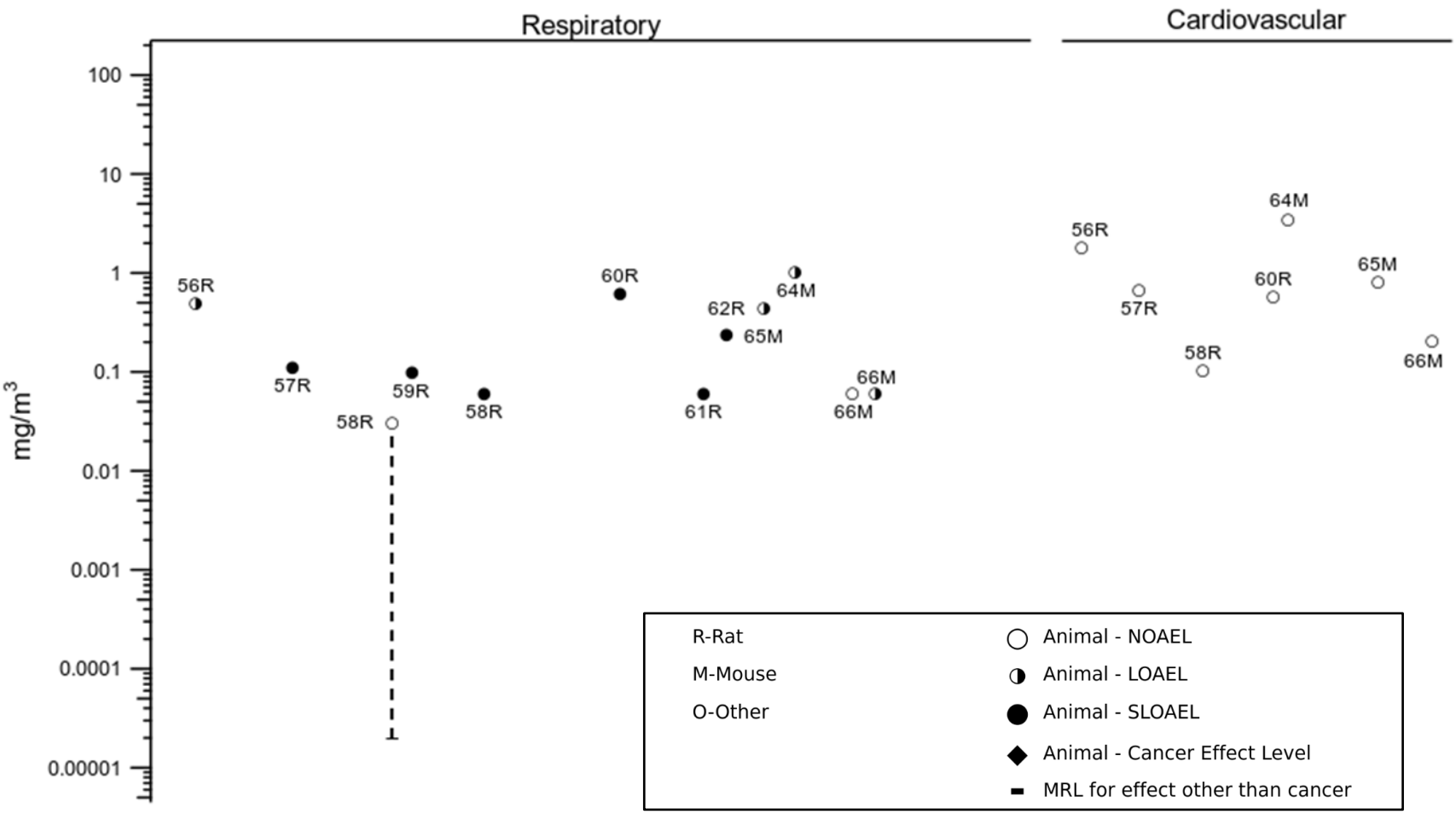

Figure 2-17. Levels of Significant Exposure to Nickel – Inhalation
Chronic (≥365 days)

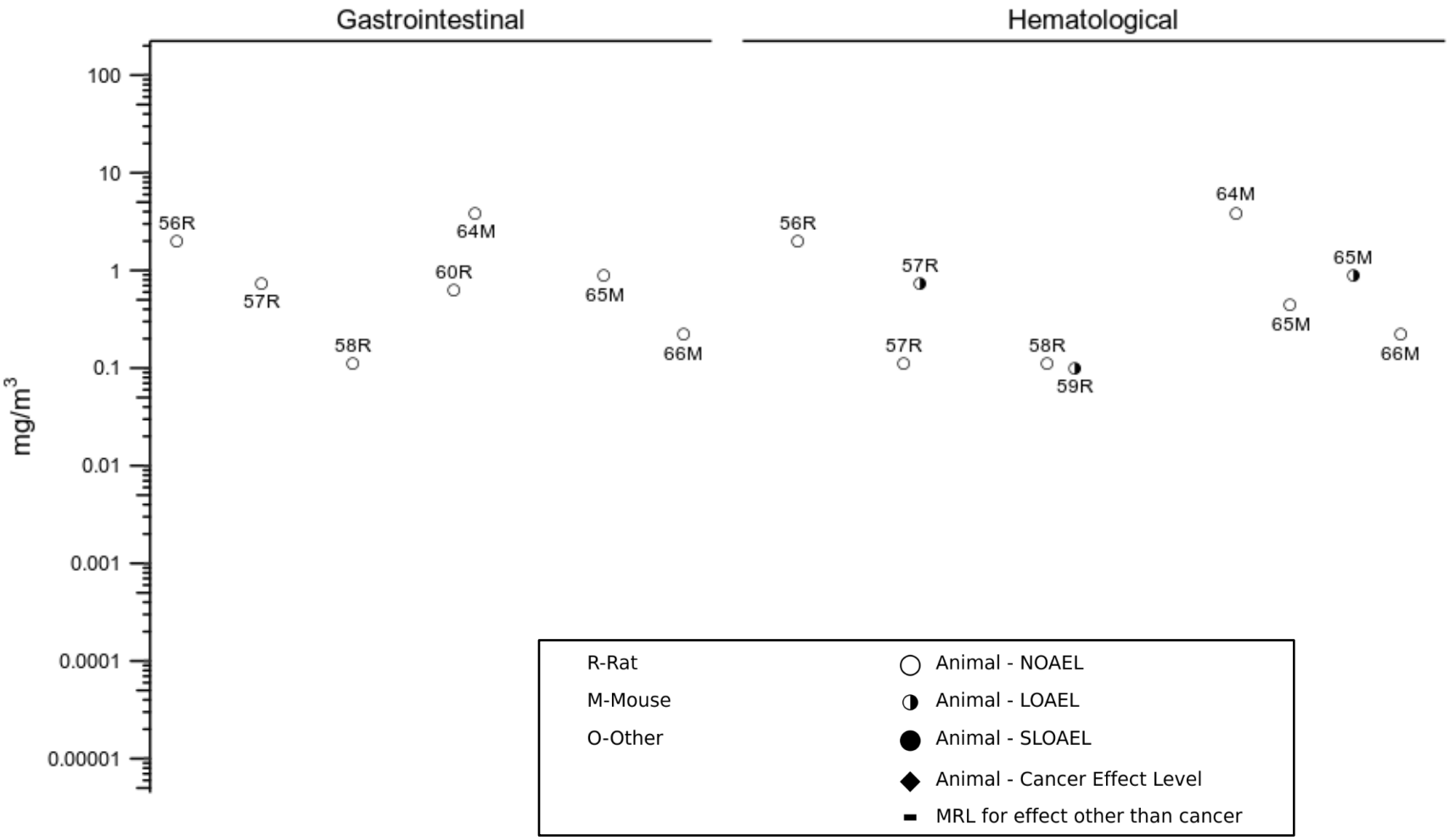

Figure 2-18. Levels of Significant Exposure to Nickel – Inhalation
Chronic (≥365 days)

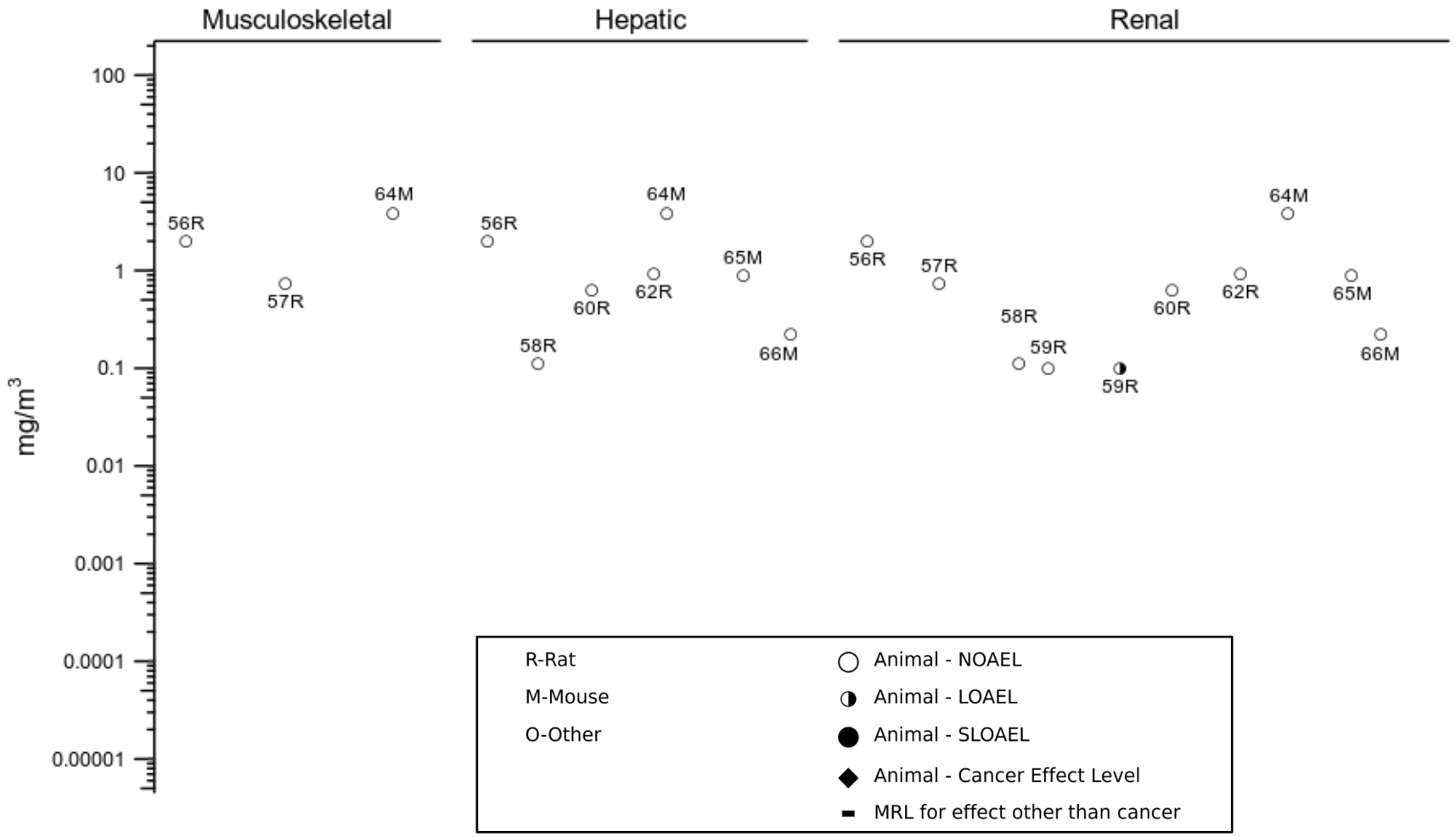

Figure 2-19. Levels of Significant Exposure to Nickel – Inhalation
Chronic (≥365 days)

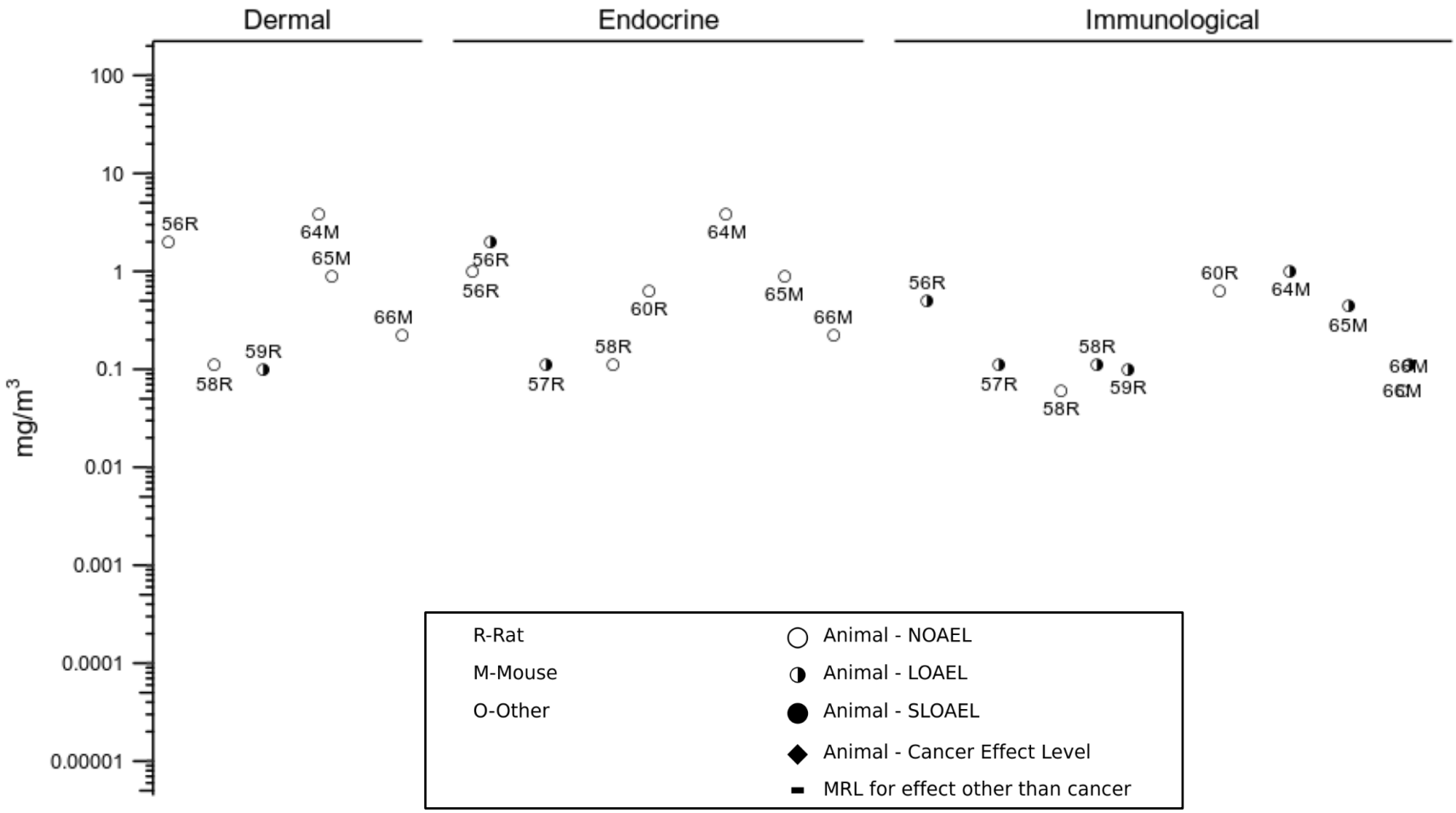

Figure 2-20. Levels of Significant Exposure to Nickel – Inhalation
Chronic (≥365 days)

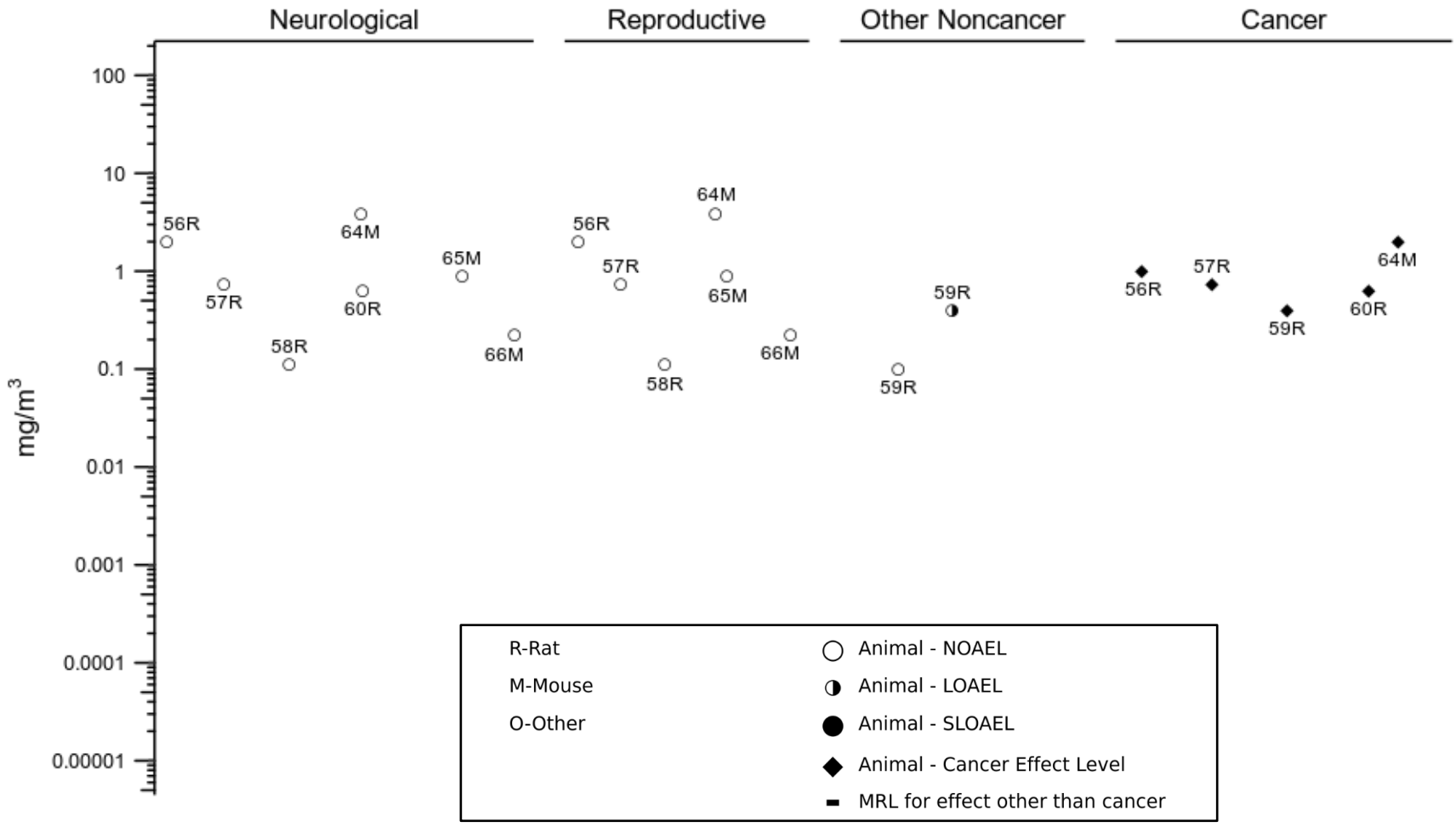

Table 2-2. Levels of Significant Exposure to Nickel – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Burrows et al. 1981									**Nickel sulfate**
1	HUMAN 22NS	2 days 2 times/day (C)	0, 0.01, 0.03	CS	Dermal	0.03			
Gawkrodger et al. 1986									**Nickel sulfate heptahydrate**
2	HUMAN 6B	Once (C)	0, 0.097	CS	Dermal		0.097 F		Allergic dermatitis in sensitized individuals
Gawkrodger et al. 1986									**Nickel sulfate heptahydrate**
3	HUMAN 20B	2 days Once/day (C)	0, 0.007, 0.043	CS	Dermal	0.043 F			
Hindsen et al. 2001									**Nickel sulfate**
4	HUMAN 9-10F	Once (C)	0, 0.014, 0.057	CS	Dermal	0.014	0.057		Dermatitis in nickel sensitive subjects
Jensen et al. 2003									**Nickel sulfate**
5	HUMAN 10F	Once (C)	0, 0.0043, 0.014, 0.057	CS	Dermal	0.014	0.057		Dermatitis in nickel sensitive subjects
Sunderman et al. 1988									**Nickel**
6	HUMAN 32M	1 day (W)	0, 7.1 - 35.7 (estimated doses)	BC CS	Gastro		7.1		Vomiting (3/20 workers), cramps (14/20), and diarrhea (4/20)
					Neuro		7.1		Giddiness (7/20 workers), headache (5/20) and weariness (6/20)
Haro et al. 1968									**Nickel acetate**
7	RAT (Fischer-344) 10M, 10F	Once (G)	66.4, 99.6, 132.8, 165.9, 199.2, 232.4, 265.6	CS GN HP	Death			116 F	Calculated LD50
								120 M	Calculated LD50

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Oller and Erexson 2007									**Nickel sulfate hexahydrate**
8	RAT (Sprague-Dawley) 6M	3 days daily (G)	0, 27.91, 55.82, 111.6, 167.4, 223.2, 279.1, 334.9, 390.7	BC CS HE LE	Death			167.4	4/6 died
					Resp		111.6		Irregular respiration in 4/6 rats
					Neuro		27.91		Hypoactivity and/or salivation
RTI 1988a, 1988b									**Nickel chloride**
9	RAT (CD) 30-32M, 30-31F	14 days (W)	F: 0, 7, 30, 55, 140; M: 0, 4, 20, 40, 140	BW CS FI GN HP WI	Death			140	7/64 died
El Sekily et al. 2020									**Nickel chloride hexahydrate**
10	MOUSE (albino) 10F	8 days daily (G)	0, 10.29, 20.59, 41.08	CS DX	Develop			10.29	Significant increase in fetal resorption and skeletal abnormalities including incomplete ossification of skull, vertebrae, ribs, sternum, fore and hind limbs, carpals, metacarpals, and phalanges; supernumerary ribs
Gray et al. 1986									**Nickel chloride**
11	MOUSE (CD-1) NS F	GD 8-12 Once daily (G)	0, 45.3	DX	Develop	45.3			
Haro et al. 1968									**Nickel acetate**
12	MOUSE (Swiss-Webster) 10M, 10F	Once (G)	66.4, 99.6, 132.8, 165.9, 199.2, 232.4, 265.6	CS HP	Death			139 F	Calculated LD50
								136 M	Calculated LD50

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
He et al. 2013									**Nickel chloride hexahydrate**
13	MOUSE (Kunning) 8M	Once (G)	0, 1.234, 12.34	BI NX	Neuro	1.234		12.34	Reduced spatial memory performance indicated by increased escape latencies 3 hours after exposure; reduced locomotor activity indicated by reduced distance traveled
					Other noncancer	1.234	12.34		Disturbance to aerobic metabolism indicated by reduced oxygen consumption, decreased superoxide dismutase activity, and decreased aconitase activity at 3 hours post-exposure
Saini et al. 2013									**Nickel chloride hexahydrate**
14	MOUSE (Swiss Albino) 10F	GD 6-13 daily	0, 11.38, 22.77, 45.55	BW DX FI LE RX	Repro			11.38	4.16% embryos resorbed/post-implantation death
					Develop			11.38	5% of offspring with microphthalmia, and 22.7% with skeletal anomalies including reduced or fused sternebrae, absence or gap between the ribs, and reduced ossification
					Other noncancer	11.38	22.77		14% reduction in food consumption
Saini et al. 2014a									**Nickel chloride hexahydrate**
15	MOUSE (Swiss Albino) 10F	GD 0-5 daily (W)	0, 11.35, 22.71, 45.68	BW DX FI LX RX WI	Neuro	45.68			
					Repro			11.35	25 and 28.5% reduction in mean number of implantation sites/dam and mean number of live fetuses/dams, respectively

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Develop		11.35		Increased incidence of skeletal anomalies (in 12% of fetuses) including reduced ossification of intraparietal skull bones, metatarsals, and phalanges, and reduced number of ribs
					Other noncancer		45.68		11% reduction in diet consumption and water intake
Saini et al. 2014b									**Nickel chloride hexahydrate**
16	MOUSE (Swiss Albino) 15F	GD 0-5 daily (G)	0, 10.29, 20.59, 41.19	BW DX RX	Repro		10.29	20.59	Reduced gestation index (75%) at 10.29 mg Ni/kg/day 16% reduction in average litter size/dam during preimplantation at 20.59 mg Ni/kg/day
					Develop	10.29	20.59	41.19	Significant 27% and 9% decrease of offspring body weight in postpartum week 1 and 6 (end of study period), respectively at 20.59 mg Ni/kg/day 11.75% offspring mortality at 41.19 mg Ni/kg/day
Saini et al. 2014b									**Nickel chloride hexahydrate**
17	MOUSE (Swiss Albino) 15F	GD 6-13 daily (G)	0, 10.29, 20.59, 41.19	BW DX RX	Repro	20.59		41.19	Reduced mean litter size/dam (24%)
					Develop		10.29	20.59	14% less offspring bodyweight at birth at 10.29 mg Ni/kg/day 9.5% offspring mortality and microphthalmia in 5% of offspring at 20.59 mg Ni/kg/day

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Saini et al. 2014b									**Nickel chloride hexahydrate**
18	MOUSE (Swiss Albino) 15F	GD 14-18 daily (G)	0, 10.29, 20.59, 41.19	BW DX RX	Repro	20.59		41.19	Reduced mean litter size/dam (27%)
					Develop	10.29		20.59	11.11% offspring mortality
Seidenberg et al. 1986									**Nickel chloride**
19	MOUSE (ICR) 28F	GD 8-12 (GW)	0, 90.6	BW DX RX	Repro	90.6			
					Develop	90.6			
Sobti and Gill 1989									**Nickel sulfate**
20	MOUSE (lacca) NS M	Once (GW)	0, 27.68	CS HP	Repro			27.68	3-fold increase in sperm head abnormalities
Sobti and Gill 1989									**Nickel nitrate**
21	MOUSE (lacca) NS M	Once (GW)	0, 23	CS HP	Repro			23	3.7-fold increase in sperm head abnormalities
Sobti and Gill 1989									**Nickel chloride**
22	MOUSE (lacca) NS M	Once (GW)	0, 43	CS HP	Repro			43	2.6-fold increase in sperm head abnormalities
Ambrose et al. 1976									**Nickel sulfate**
23	DOG (Beagle) 3M, 3F	3 days (F)	0, 2.5, 25, 62.5	BW CS FI GN HP OW UR	Gastro	25	62.5		Vomiting (6/6 dogs)

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
INTERMEDIATE EXPOSURE									
Santucci et al. 1994									**Nickel sulfate**
24	HUMAN 8F	91-178 days (Ni-sensitized individuals) (W)	0.01-0.03	CS	Dermal	0.02			
Adeyemi and Elebiyo 2014									**Nickel sulfate**
25	RAT (Wistar) 5M	21 days daily (G)	0, 7.585	BC BI BW OW	Bd wt	7.585			
					Renal		7.585		Increased plasma creatinine and urea and renal cell alterations including swollen tubules and mild necrosis
Adeyemi et al. 2017									**Nickel sulfate**
26	RAT (Wistar) 6M	21 days daily (GW)	7.585	BC BI BW HE HP OW	Cardio		7.585		Increased atherogenic index
					Hemato		7.585		Altered blood chemistry (reduced plasma protein and GSH, increased MDA, TC, TAG, and LDL-C).
					Hepatic		7.585		Significantly increased liver enzyme levels: ALT (>300%), AST (>400%), and ALP (>100%) with liver inflammation and cellular degeneration
Ambrose et al. 1976									**Nickel sulfate**
27	RAT (Wistar) 30M, 30F	3-generation study; F0 and F1 generation each exposed for 11 weeks (F)	0, 22.5, 45, 90	BW CS DX GN HP RX	Bd wt	90 F			
						45 M	90 M		13% decrease in body weight of F1 generation compared to controls
					Repro			22.5	Increased incidence in number of stillborn (23 stillborn) in F1 generation (8 stillborn among controls)
					Develop	90			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
American Biogenics Corp 1988									**Nickel chloride**
28	RAT (Sprague-Dawley) 30M, 30F	91 days daily (GW)	0, 1.2, 8.6, 25	BC BW CS HP LE	Death			25	100% mortality due to exposure by study day 76 for males and study day 78 for females
					Bd wt	1.2 F	8.6 F		12% decrease in body weight gain
					Resp		8.6		Pneumonitis
					Cardio	8.6			
					Gastro	8.6		25	Ulcerative gastritis, enteritis, and abnormal intestinal contents
					Hemato	1.2 F	8.6 F		Increased platelet count
					Hepatic	8.6			
					Renal	8.6			
					Dermal	8.6			
					Ocular	8.6			
					Neuro	1.2		8.6	Ataxia, prostration, hypothermia
					Other noncancer	1.2 F	8.6 F		Decreased blood glucose level
Heim et al. 2007									**Nickel sulfate hexahydrate**
29	RAT (Fischer-344) NS M, NS F	90 days (G)	0, 11.16, 16.74, 22.32, 27.91, 33.49	BW HP	Bd wt	22.32 F	27.91 F		~10% decrease in body weight
						11.16 M	16.74 M		~12% decrease in body weight
Kakela et al. 1999									**Nickel chloride**
30	RAT (Wistar) 6M	28 or 42 days before copulation Daily (W)	0, 3.6	DX HP RX	Repro			3.6	Significantly decreased gestation index (73.5% less compared to controls) and decreased litter size by lactation day 21 (86% less than controls)

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Develop			3.6	Structural abnormalities in pups that died including underdeveloped posteriors of the bodies, slow movement, and disproportionately large heads
Kakela et al. 1999									**Nickel chloride**
31	RAT (Wistar) 6F	62 days: 14 or 100 days before copulation through LD 48 daily (W)	0, 1.3, 4.0, 13	DX RX	Repro			13	Significantly decreased litter size by lactation day 21 (56.5% less than controls)
					Develop			13	Structural abnormalities in pups that died including underdeveloped posteriors of the bodies, slow movement, and disproportionately large heads; lower relative kidney and liver weights
Kakela et al. 1999									**Nickel chloride**
32	RAT (Wistar) 6M, 6F	28-76 days daily (W)	M: 0, 3.6; F: 0, 4.0	DX RX	Repro			3.6	Significantly decreased litter size by lactation day 21 (71% less than controls); 44% pup survival
					Develop			3.6	Structural abnormalities in pups that died including underdeveloped posteriors of the bodies, slow movement, and disproportionately large heads
Kamal et al. 2012									**Nickel sulfate hexahydrate**
33	RAT (albino) 6M	28 days daily (W)	0, 3.81, 10.00	BI BW FI	Hepatic		3.81		Increased ALT (248%), AST (56%), MDA (29%), and decreased SOD (29%), GST (20%)

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Mahmoud et al. 2011									**Nickel sulfate heptahydrate**
34	RAT (albino) 4M	21 days daily (W)	0, 3.563	BC BI BW CS FI WI	Hepatic		3.563		Altered liver enzymes, including increased MDA content (30%), serum ALT (248%) and AST (56%), and reduced SOD (30%) activity
					Renal		3.563		Increased SOD (25%) and GSH (60%) activity in kidney tissue
					Other noncancer		3.563		13.5% and 8% reduction in fluid and food intake, respectively
Obone et al. 1999									**Nickel sulfate**
35	RAT (Sprague-Dawley) 8M	13 weeks daily (W)	0, 5.75, 14.4, 28.8	BI BW HP LE OW	Bd wt	28.8			
					Resp		5.75		66% decrease of alkaline phosphatase activity in bronchioalveolar lavage fluid compared to controls
					Cardio	28.8			
					Gastro	28.8			
					Hepatic	28.8			
					Renal	5.75	14.4		Decreased urine volume and urine glucose
					Immuno	5.75	14.4		63 and 80% increase in absolute %CD8+ T-cells in the spleen and thymus; 34% decrease of CD4:CD8 ratio compared to controls
					Neuro	28.8			
					Repro	28.8			
RTI 1988a, 1988b									**Nickel chloride**
36		PO generation exposure		BW CS FI GN HP WI	Resp	4 M	20 M		Histiocytic cellular infiltration in lungs in F1 generation

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
	RAT (CD) 30-32M, 30-31F	began 11 weeks prior to breeding; total exposure: F: 27-30 weeks M: 21-24 weeks (W)	F: 0, 7, 30, 55; M: 0, 4, 20, 40		Renal	55 F			
					Repro	7 F	30 F		Increased gestation length in first P0 pregnancy
					Develop	7 F		30 F	Increased mortality in F1b rats on PND 22-42
Smith et al. 1993									**Nickel chloride**
37	RAT (Long-Evans) 34F	11 weeks (breeding-lactation) 2 litters (W)	0, 1.3, 6.8, 31.6	BC BW CS DX FI WI	Bd wt	31.6			
					Endocr	6.8	31.6		21% decreased prolactin
					Repro	31.6			
					Develop			1.3	Decreased pup survival
Springborn Laboratories 2000a									**Nickel sulfate hexahydrate**
38	RAT (Sprague-Dawley) 28M, 28F	18 weeks Daily F1 generation (GW)	0, 0.22, 0.56, 1.1, 2.2	BW CS DX FI GN HP LE OW RX	Bd wt	2.2			
					Resp	2.2			
					Cardio	2.2			
					Gastro	2.2			
					Hepatic	2.2 F			
						0.56 M	1.1 M		Significant 7.3% decrease in relative liver weight
					Renal	2.2			
					Dermal	2.2			
					Endocr	2.2			
					Immuno	2.2			
					Neuro	2.2			
					Repro	2.2			
					Develop	2.2			

Table 2-2. Levels of Significant Exposure to Nickel – 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	2.2			No treatment-related changes in food consumption, hair loss, or discolorations
Springborn Laboratories 2000a									**Nickel sulfate hexahydrate**
39	RAT (Sprague-Dawley) 28M, 28F	16 weeks Daily F0 generation (GW)	0, 0.22, 0.56, 1.1, 2.2	BW CS DX FI GN HP LE OW RX	Bd wt	2.2			
					Resp	2.2			
					Gastro	2.2			
					Hemato	2.2			
					Hepatic	2.2 F			
						1.1 M	2.2 M		Significant 9% decrease in relative liver weight
					Renal	2.2			
					Dermal	2.2			
					Endocr	2.2			
					Immuno	2.2			
					Neuro	2.2			
					Repro	2.2			
					Other noncancer	2.2			No treatment-related changes in food consumption, hair loss, or discolorations
Springborn Laboratories 2000a									**Nickel sulfate hexahydrate**
40	RAT (Sprague-Dawley) 325-394B	exposure in utero and during lactation; both parents exposed (GW)	0, 0.22, 0.56, 1.1, 2.2	DX	Develop	2.2			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Springborn Laboratories 2000b									**Nickel sulfate hexahydrate**
41	RAT (Sprague-Dawley) 8M, 8F	F1 generation began on PND 22 for 1, 2, or 3 weeks (GW)	0, 2.2, 4.5. 6.7, 11.2, 16.7	BW CS DX GN LE	Develop	4.5		6.7	Significantly increased incidence of stillborn pups on lactation day 0 (23 dead vs 1 dead in controls) and significantly reduced mean live litter size (29%)
Springborn Laboratories 2000b									**Nickel sulfate hexahydrate**
42	RAT (Sprague-Dawley) 8M, 8F	Began 2 weeks before mating to LD 21 for F0 generation Daily (GW)	0, 2.2, 4.5. 6.7, 11.2, 16.7	CS BW FI GN LE RX WI	Bd wt	16.7			
					Resp	16.7			
					Gastro	16.7			
					Hepatic	16.7			
					Renal	16.7			
					Endocr	16.7			
					Neuro	16.7			
					Repro	4.5		6.7	Significantly increased post-implantation loss (475% more than controls)
					Other noncancer	16.7			No exposure-related changes in food or water intake
Springborn Laboratories 2002									**Nickel sulfate**
43	RAT (Fischer-344) 10M, 10F	90 days daily (GW)	M: 0, 11, 17, 22, 13, 13; F: 0, 11, 17, 22, 28, 33	CS HP	Bd wt	11 M	17 M		12.2% decrease in final body weight
					Resp	22 M			
					Cardio	22 M			
					Gastro	22 M			
					Hepatic	22 M			
					Renal	22 M			
					Endocr	22 M			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Vyskocil et al. 1994b									**Nickel sulfate**
44	RAT (Wistar) 10M, 10F	3 or 6 months (W)	0, M:6.9, F:7.6	BW UR	Bd wt	7.6 F			
					Renal		7.6 F		Increased urinary albumin
Weischer et al. 1980									**Nickel chloride**
45	RAT (Wistar) 10M	28 days (W)	0, 0.23, 0.49, 0.97	BC BW OW WI	Bd wt			0.23	20% decreased body weight gain
					Hemato	0.23	0.49		Increased leukocytes (36%)
					Hepatic	0.97			
					Renal		0.23		Decreased urea (15%)
Whanger 1973									**Nickel acetate**
46	RAT (OSU brown) 6M	6 weeks (F)	0, 5, 25, 50	BI BW HE	Bd wt	5		25	88% decrease in body weight gain
					Hemato	50			
Berman and Rehnberg 1983									**Nickel chloride**
47	MOUSE (CD-1) 12-24F	GD 2-17 (W)	0, 80, 160	DX RX	Develop	80		160	Increased spontaneous abortions
Dahdouh et al. 2016									**Nickel sulfate**
48	MOUSE (Swiss albino) 8M	28 days daily (F)	0, 0.036	BC BI BW FI HE HP OW WI	Hemato		0.04		Significant changes in blood composition including 25, 26, and 24% reductions in RBCs, PVC%, and hemoglobin, and 33% increase WBC count all compared to controls
					Renal			0.04	Proximal tubule degeneration with tubular necrosis and inflammation

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Dieter et al. 1988									**Nickel sulfate**
49	MOUSE (B6C3F1) 10F	180 days daily (W)	0, 44, 108, 150	BI BW HP OW WI	Bd wt	44	108	150	Body weight 10% lower than controls at 108 mg Ni/kg/day Body weight 26% lower than controls at 150 mg Ni/kg/day
					Hepatic	150			
					Renal	44	108		Hyaline casts, loss of tubular epithelial cells
					Immuno		44		Mild thymic atrophy, impaired B-cell immune function, decreased granulocyte macrophage progenitor cell levels
Gathwan et al. 2013									**Nickel chloride**
50	MOUSE (BALB/c) 5M	40 days daily (NS)	0, 0.905, 3.714, 7.246	BI BW FI HP OW WI	Bd wt	7.246			
					Hepatic		0.905	7.246	Diffused cytoplasm and damaged nuclei in hepatic cells at 0.905 mg Ni/kg/day Hepatocellular degeneration with hypertrophy of nuclei and blood in the central canal of the liver at 7.246 mg Ni/kg/day
Ilback et al. 1994									**Nickel chloride**
51	MOUSE (BALB/c) 8F	10-11 weeks (W)	0, 20.3	BW HP LE OF	Immuno		20.3		Enhanced inflammatory response in the hearts of mice challenged with coxsackie virus B3
Pandey and Srivastava 2000									**Nickel chloride**
52	MOUSE (NS) 6M	35 days 5 days/week (GW)	0, 1.2, 2.5, 4.9	RX	Repro	1.2		2.5	24 and 25% decreased in sperm motility and count, respectively; increased sperm abnormalities

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Pandey and Srivastava 2000									**Nickel sulfate**
53	MOUSE (NS) 6M	35 days 5 days/week (GW)	0, 1.1, 2.2, 4.5	RX	Repro	1.1		2.2	12.5 and 15% decrease of sperm count and motility, respectively; significant dose-related increase in sperm head, tail, and neck abnormalities in 24% of mice
Pandey et al. 1999									**Nickel sulfate**
54	MOUSE (Swiss) 20M	35 days 5 days/week (GW)	0, 2.2	DX RX	Repro			2.2	Significant post-implantation loss (3.33% in controls vs 19.20% in treated)
Pandey et al. 1999									**Nickel sulfate**
55	MOUSE (Swiss) 20M	35 days 5 days/week (GW)	0, 1.1, 2.2	BI BW HP OW RX	Bd wt	2.2			
					Repro			1.1	7% decrease in sperm motility and 37% decrease in total sperm count; significantly reduced relative weight of testis (14%), seminal vesicle (30%), and prostate gland (25%); 117% increase in percent morphological sperm abnormalities
Toman et al. 2012									**Nickel chloride**
56	MOUSE (ICR) 5M	3-12 weeks daily (F)	0, 4.53	BW CS HP LE OW	Repro			4.53	Degeneration of seminiferous epithelium, decrease in relative volume of germinal epithelium, interstitium, blood vessels and increased relative volume of lumen, empty spaces in the epithelium and whole tubules of testes

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
CHRONIC EXPOSURE									
Heim et al. 2007									**Nickel sulfate hexahydrate**
57	RAT (Fischer-344) 60M, 60F	2 years (104 weeks) daily (G)	0, 2.232, 6.698, 11.16	BC BW CS FI GN HE LE	Death			2.232 F	Exposure-response trend in mortality, 33% mortality
					Bd wt	6.698 F	11.16 F		10% reduction in body weight
						2.232 M	6.698 M		11% reduction in bodyweight
					Hemato	11.16			
Ambrose et al. 1976									**Nickel sulfate**
58	DOG (Beagle) 3M, 3F	2 years (F)	0, 2.5, 25, 62.5	BW CS FI GN HP OW UR	Bd wt	25	62.5		10% decrease in body weight gain
					Resp	25		62.5	Cholesterol granulomas, emphysema, bronchiectasis
					Cardio	62.5			
					Gastro	62.5			
					Hemato	25	62.5		Unspecified decrease of hematocrit and hemoglobin levels suggestive of simple hypochromic anemia
					Musc/skel	62.5			
					Hepatic	62.5			
					Renal	25	62.5		Polyuria in 2/6 dogs, increased kidney weight
					Dermal	62.5			
					Endocr	62.5			
					Immuno	62.5			
					Neuro	62.5			
					Repro	62.5			

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

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.

ALP = Alkaline phosphatase; ALT = alanine transaminase; AST = aspartate transaminase; B = both sexes; Bd wt and BW= body weight; BC = serum (blood) chemistry; BI =biochemical changes; (C) = capsule; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; (F) = dietary exposure; F= female(s); FI = food intake; (G) = gavage; (GW) = gavage with aqueous vehicle); Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; GSH = glutathione; GST = glutathione-s-transferase; HE = hematological; HP = histopathology; Immuno = immunological; LD50 = dose producing 50% death; LDL-C = low-density lipoprotein cholesterol; LE = lethality; LOAEL = lowest-observed-adverse-effect-level; M = male(s); MDA = malondialdehyde; Musc/skel = musculoskeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect-level; NS = not specified; NX = neurological function; OW = organ weight; PVC =packed cell volume; RBCs = red blood cells; Repro = Reproductive; Resp = respiratory; RX = reproductive function; SLOAEL = serious lowest-observed-adverse-effect-level; SOD = superoxide dismutase; TAG = triacylglycerol; TC = total cholesterol; UR = urinalysis; (W) = drinking water; WBC = white blood cells; WI = water intake.

Figure 2-21. Levels of Significant Exposure to Nickel – Oral
Acute (≤14 days)

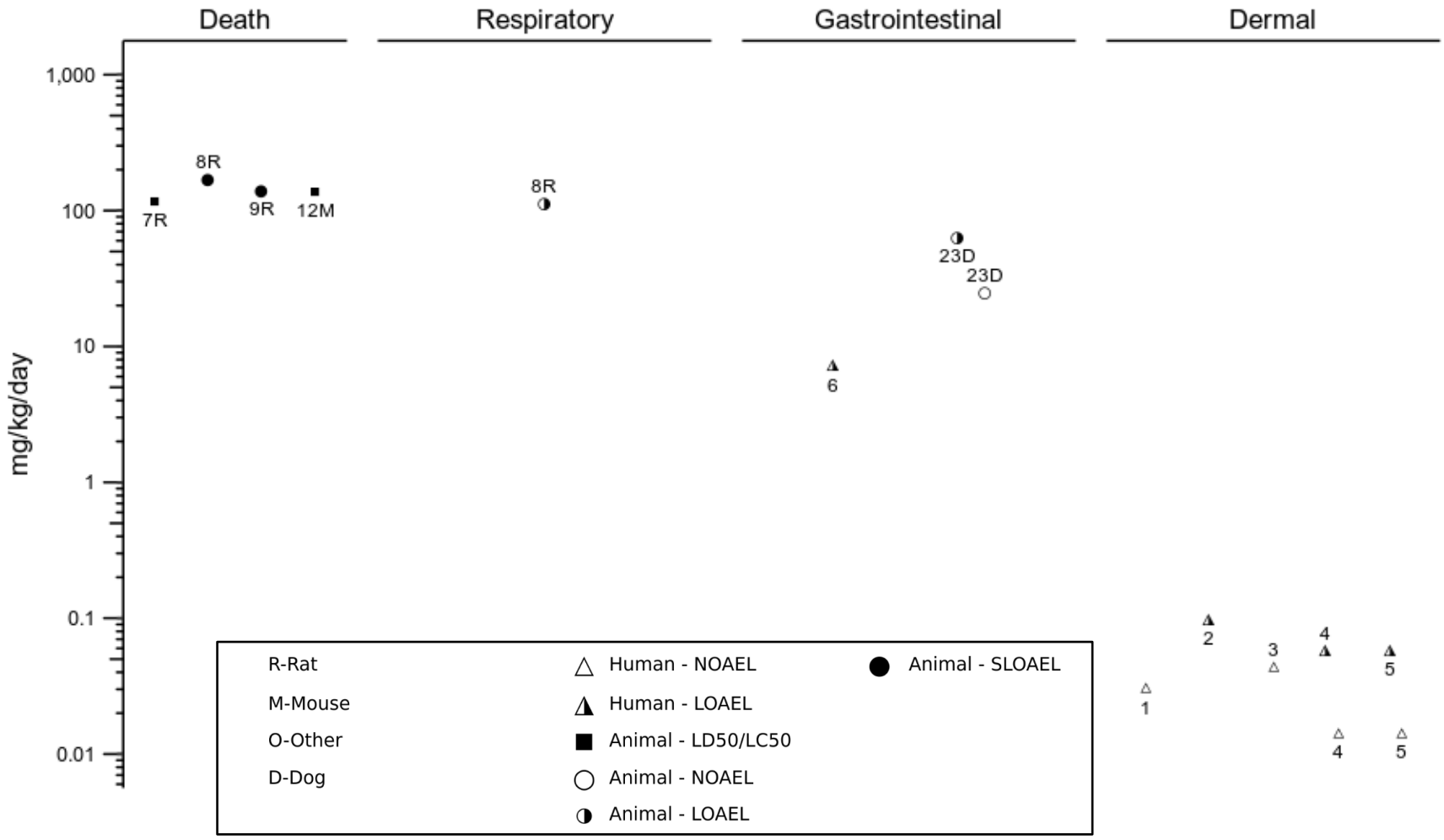

Figure 2-22. Levels of Significant Exposure to Nickel – Oral
Acute (≤14 days)

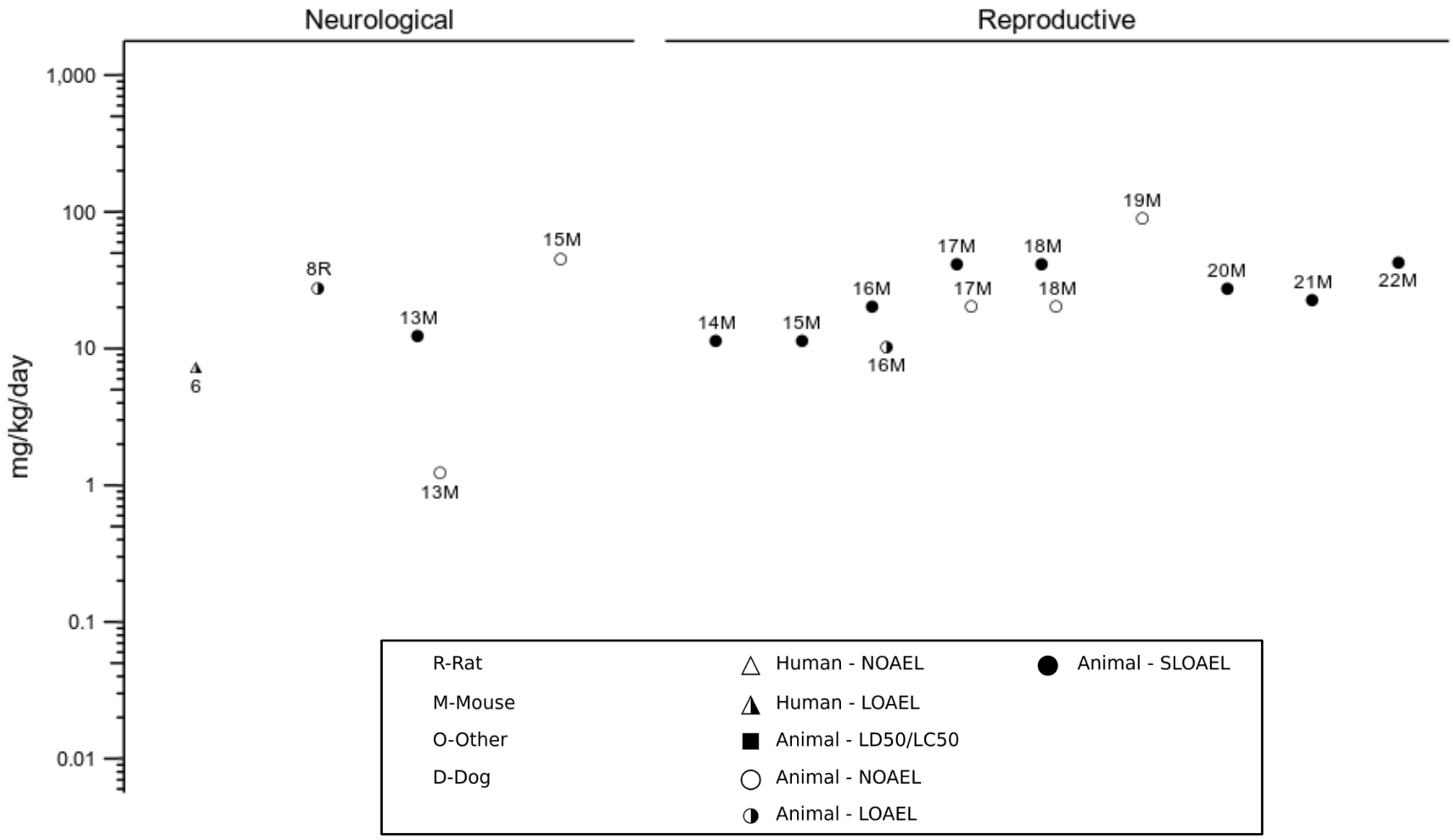

Figure 2-23. Levels of Significant Exposure to Nickel – Oral
Acute (≤14 days)

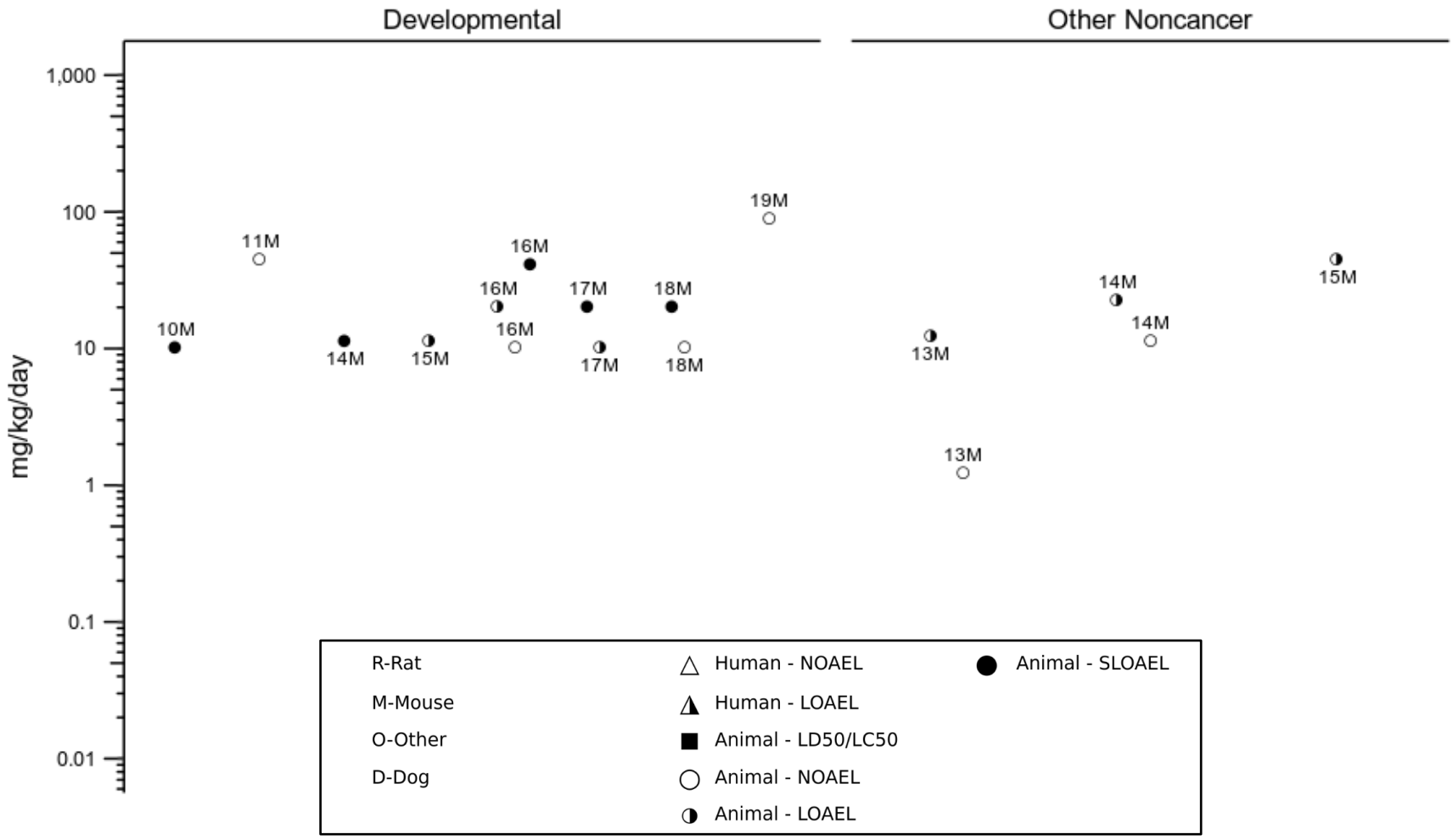

Figure 2-24. Levels of Significant Exposure to Nickel – Oral
Intermediate (15-364 days)

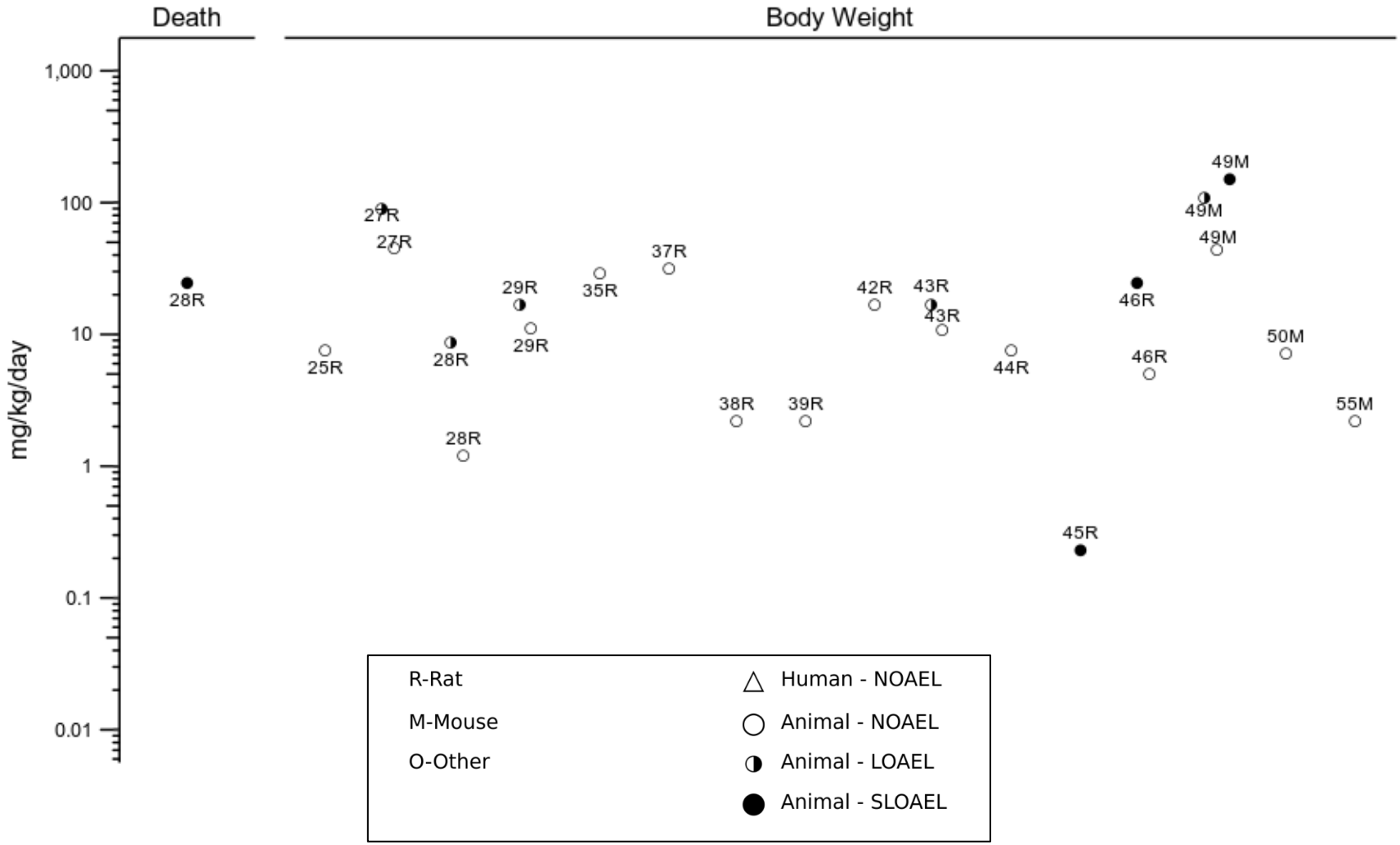

Figure 2-25. Levels of Significant Exposure to Nickel – Oral
Intermediate (15-364 days)

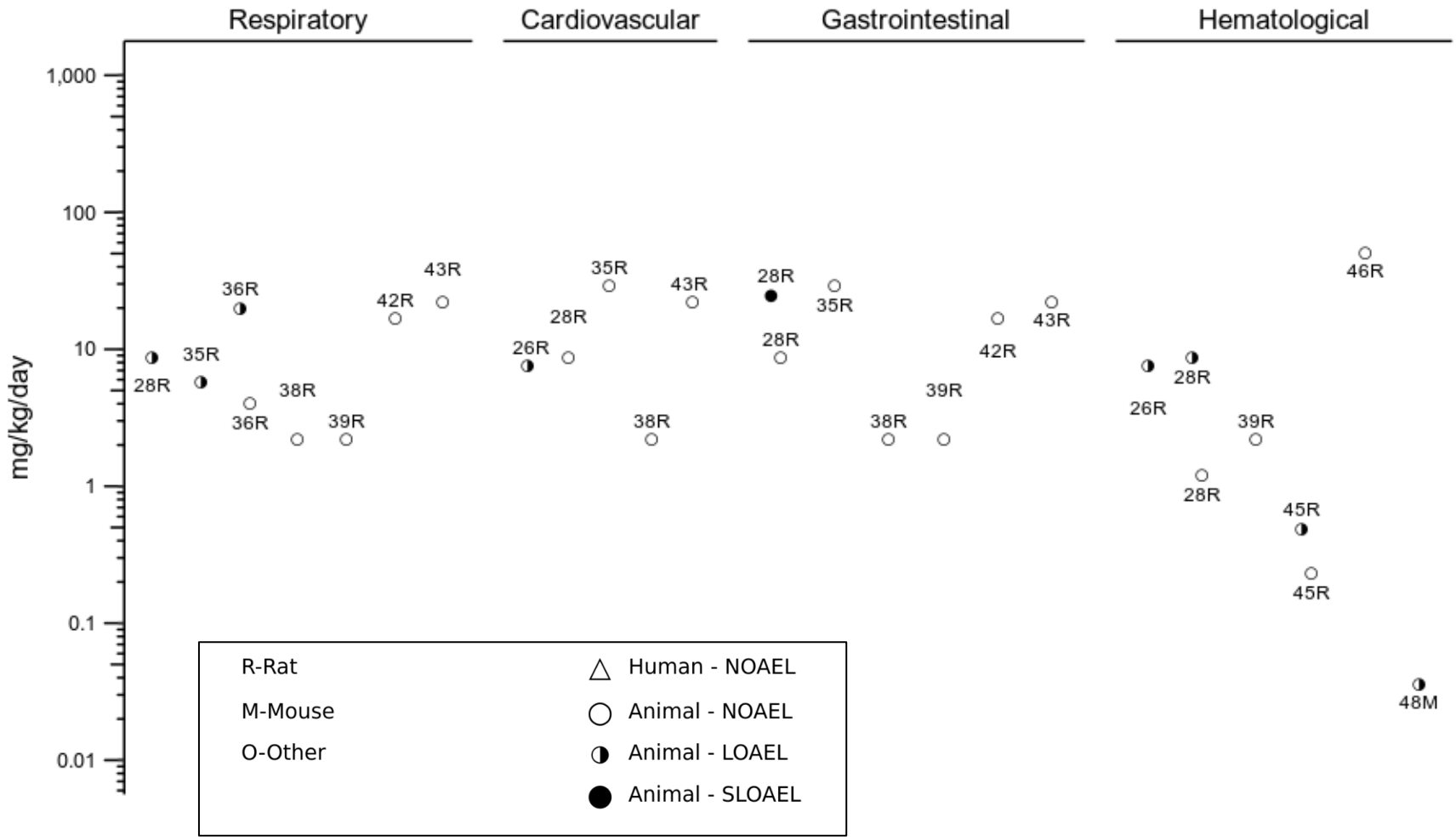

Figure 2-26. Levels of Significant Exposure to Nickel – Oral
Intermediate (15-364 days)

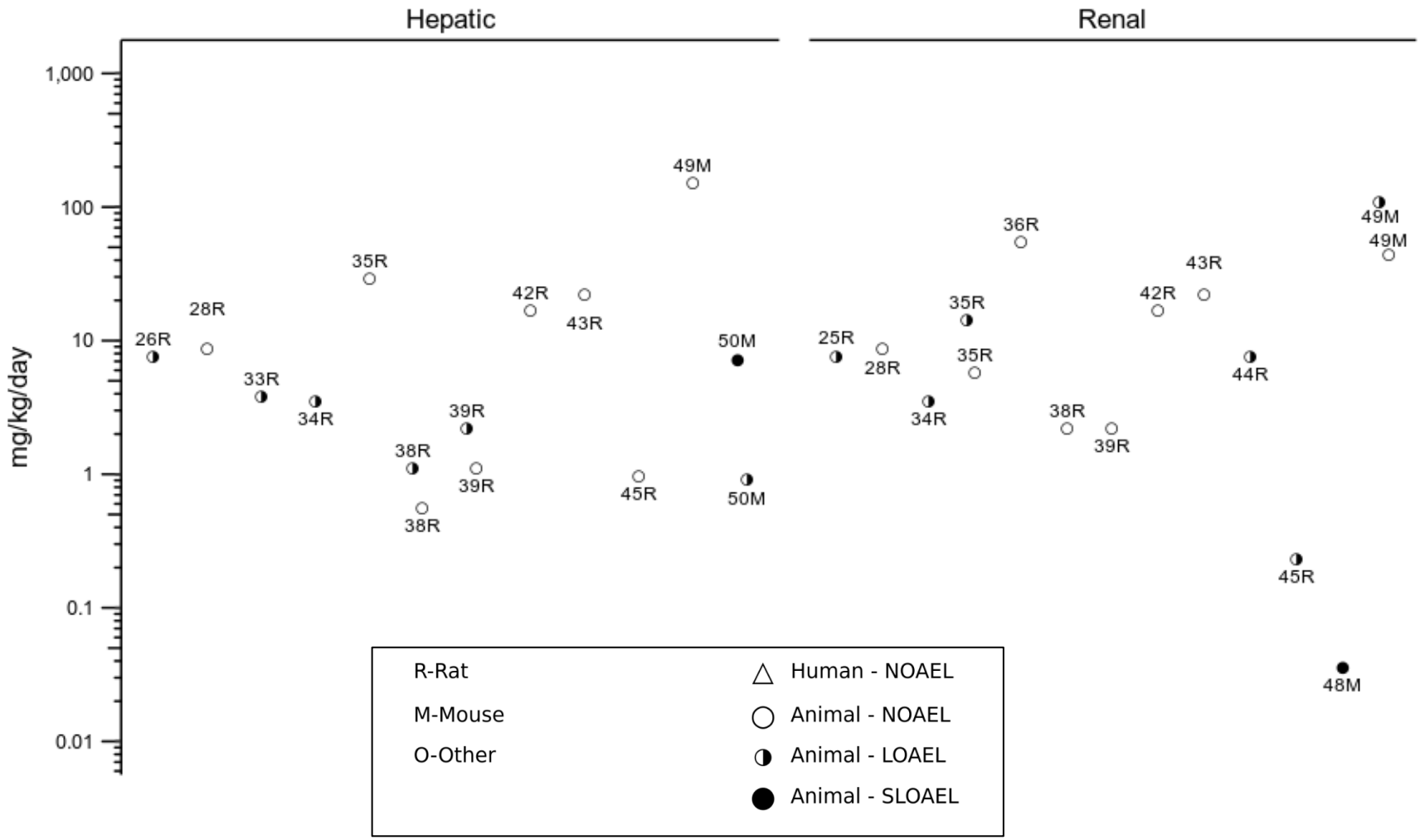

Figure 2-27 Levels of Significant Exposure to Nickel – Oral

Intermediate (15-364 days)

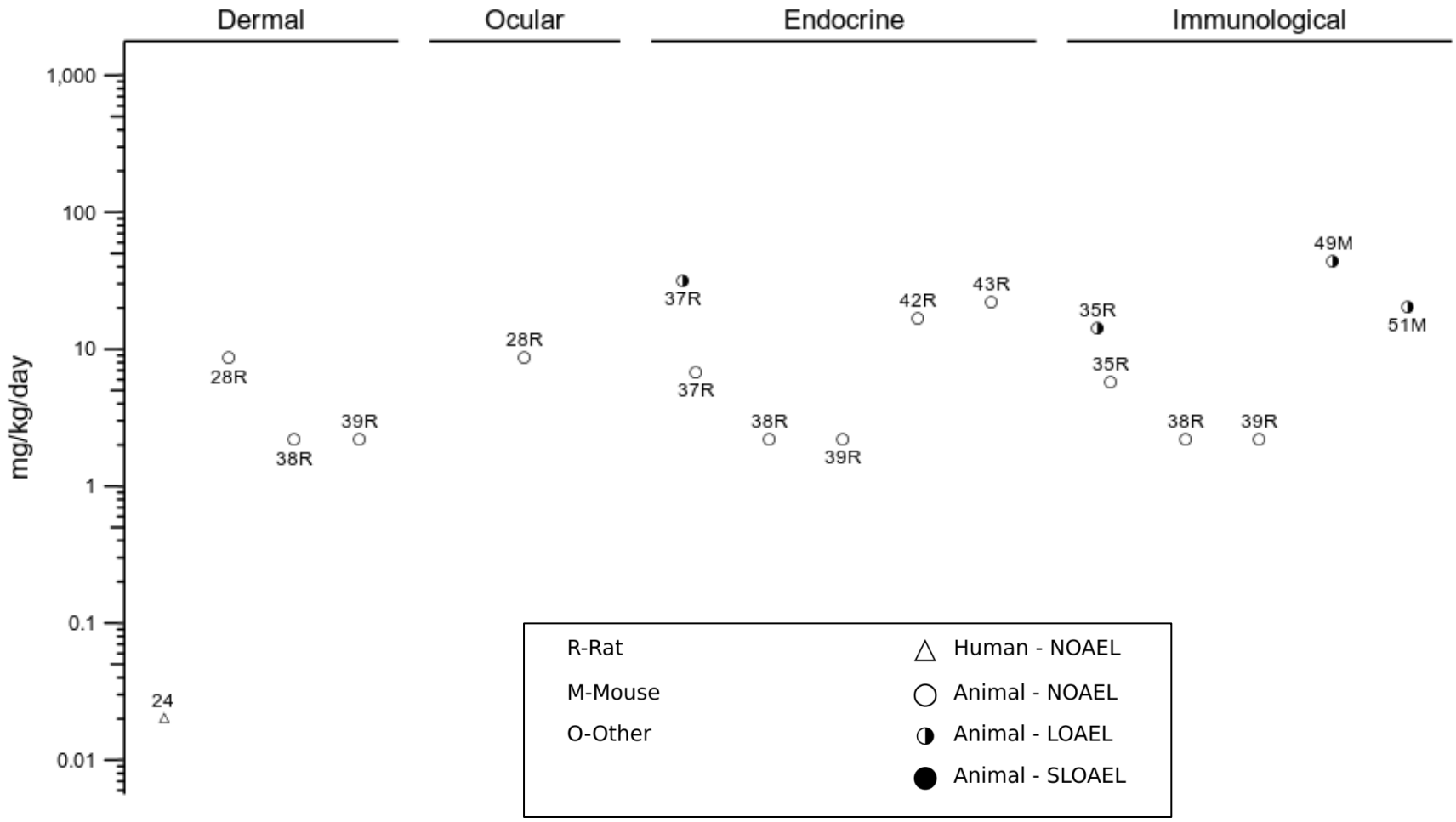

Figure 2-28 Levels of Significant Exposure to Nickel – Oral

Intermediate (15-364 days)

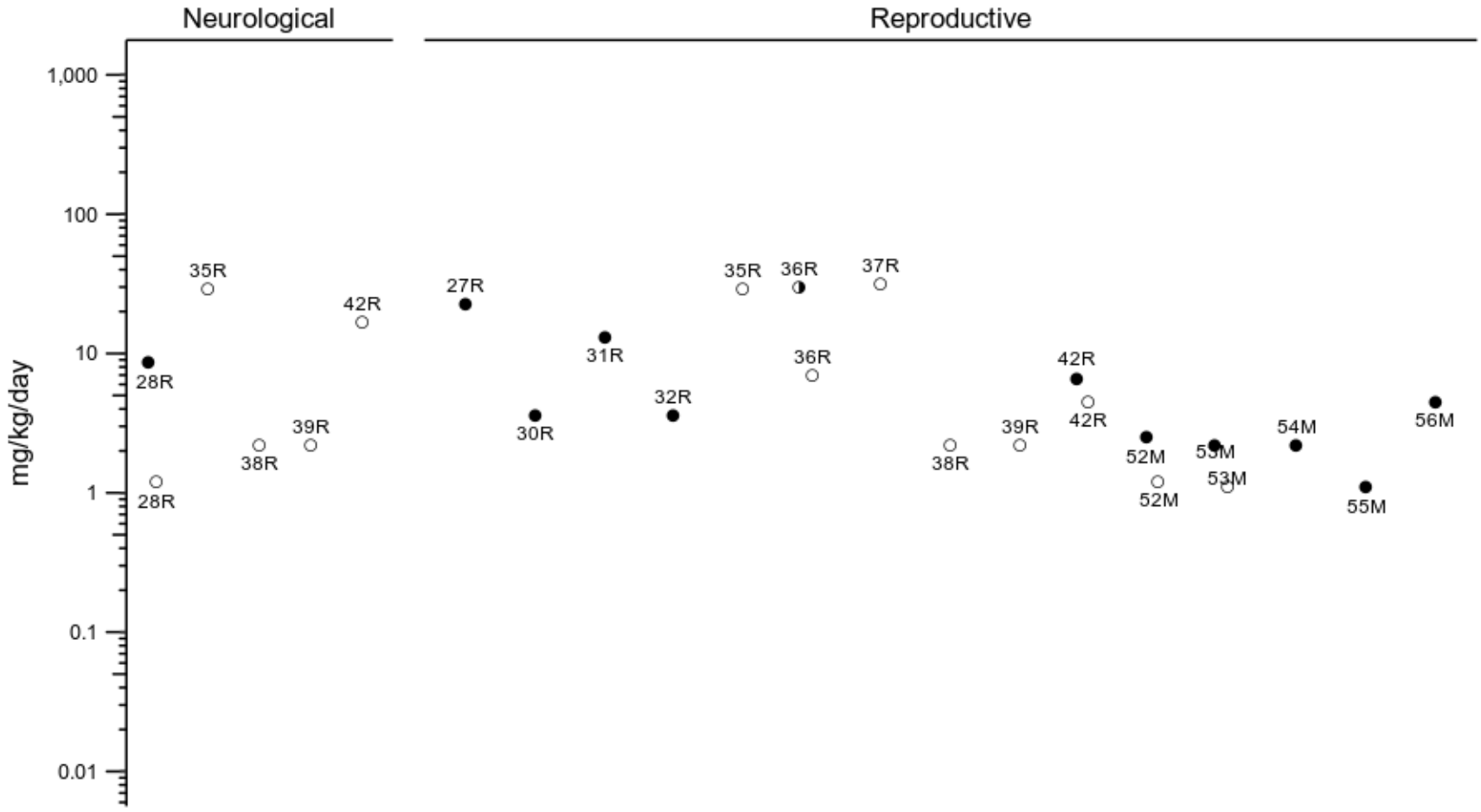

Figure 2-29 Levels of Significant Exposure to Nickel – Oral
Intermediate (15-364 days)

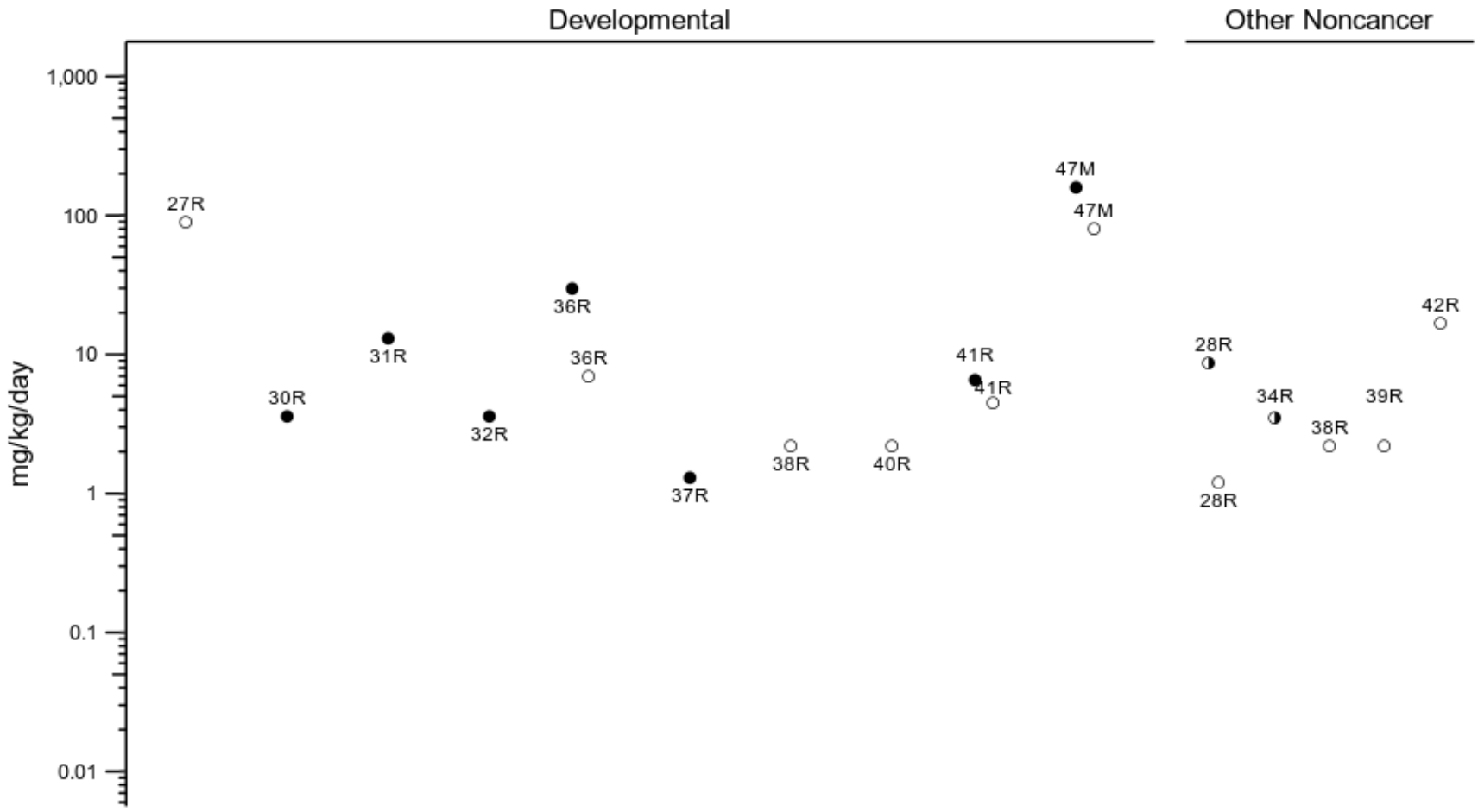

Figure 2-30. Levels of Significant Exposure to Nickel – Oral
Chronic (≥365 days)

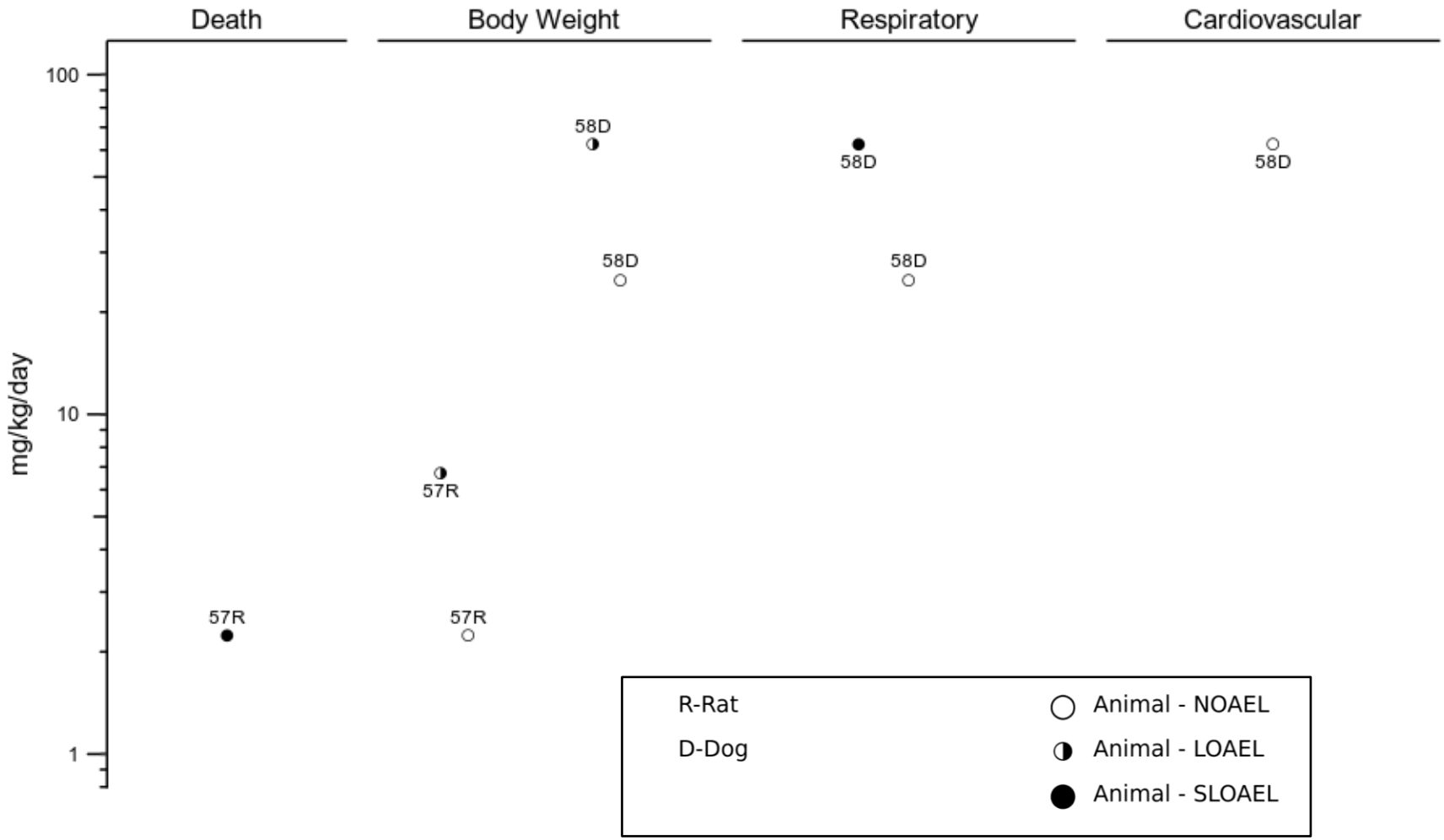

Figure 2-31. Levels of Significant Exposure to Nickel – Oral
Chronic (≥365 days)

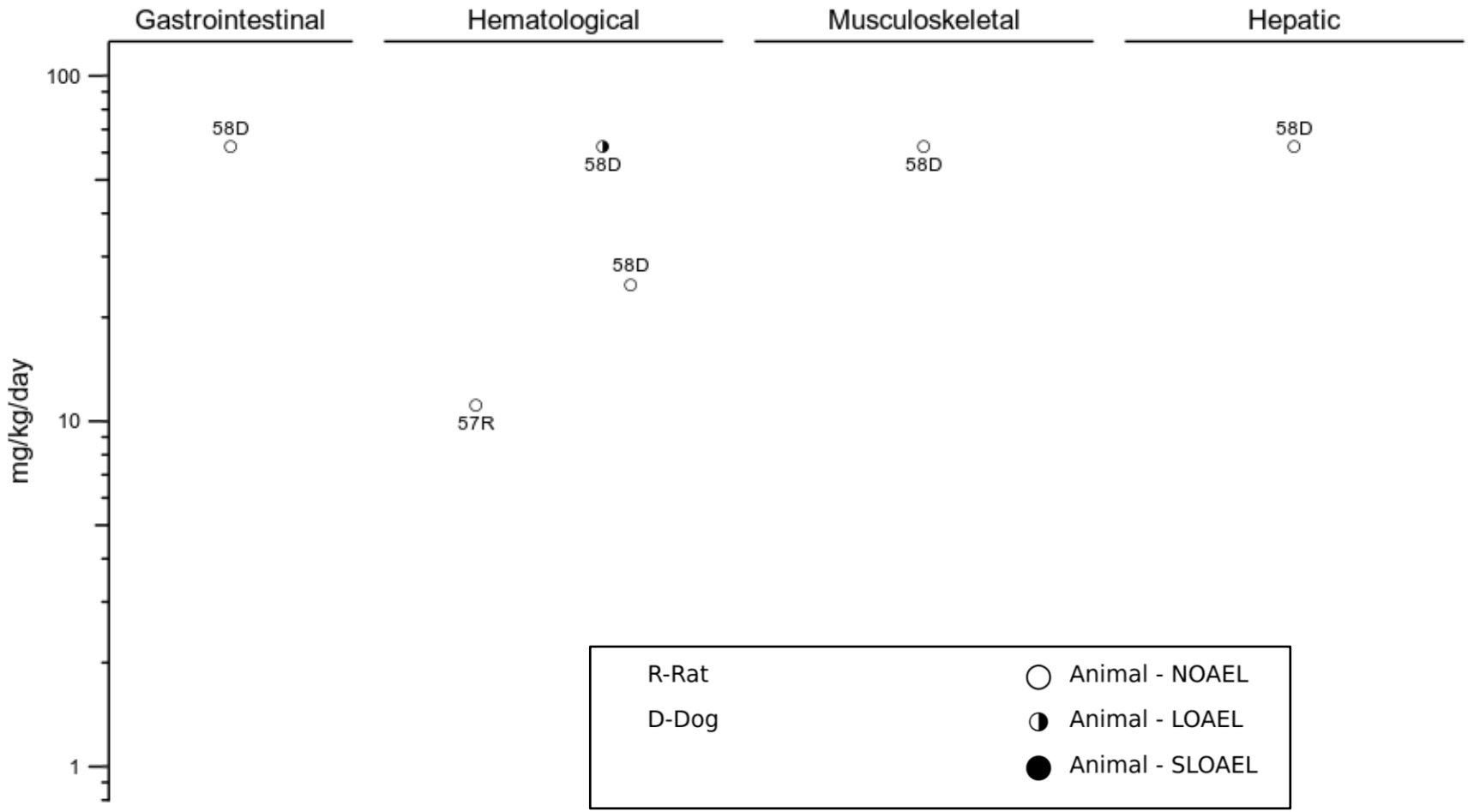

Figure 2-32. Levels of Significant Exposure to Nickel – Oral
Chronic (≥365 days)

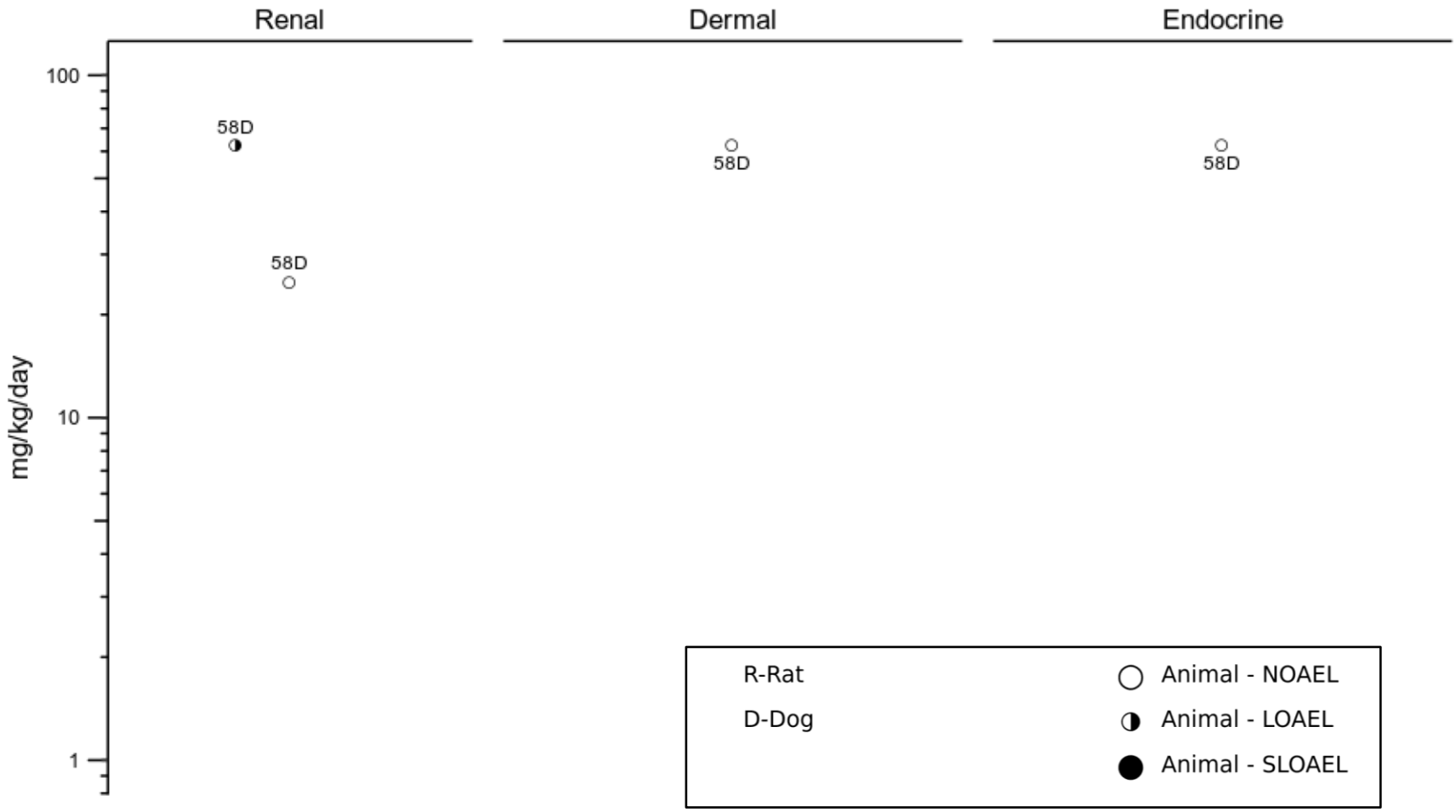

Figure 2-33. Levels of Significant Exposure to Nickel – Oral
Chronic (≥365 days)

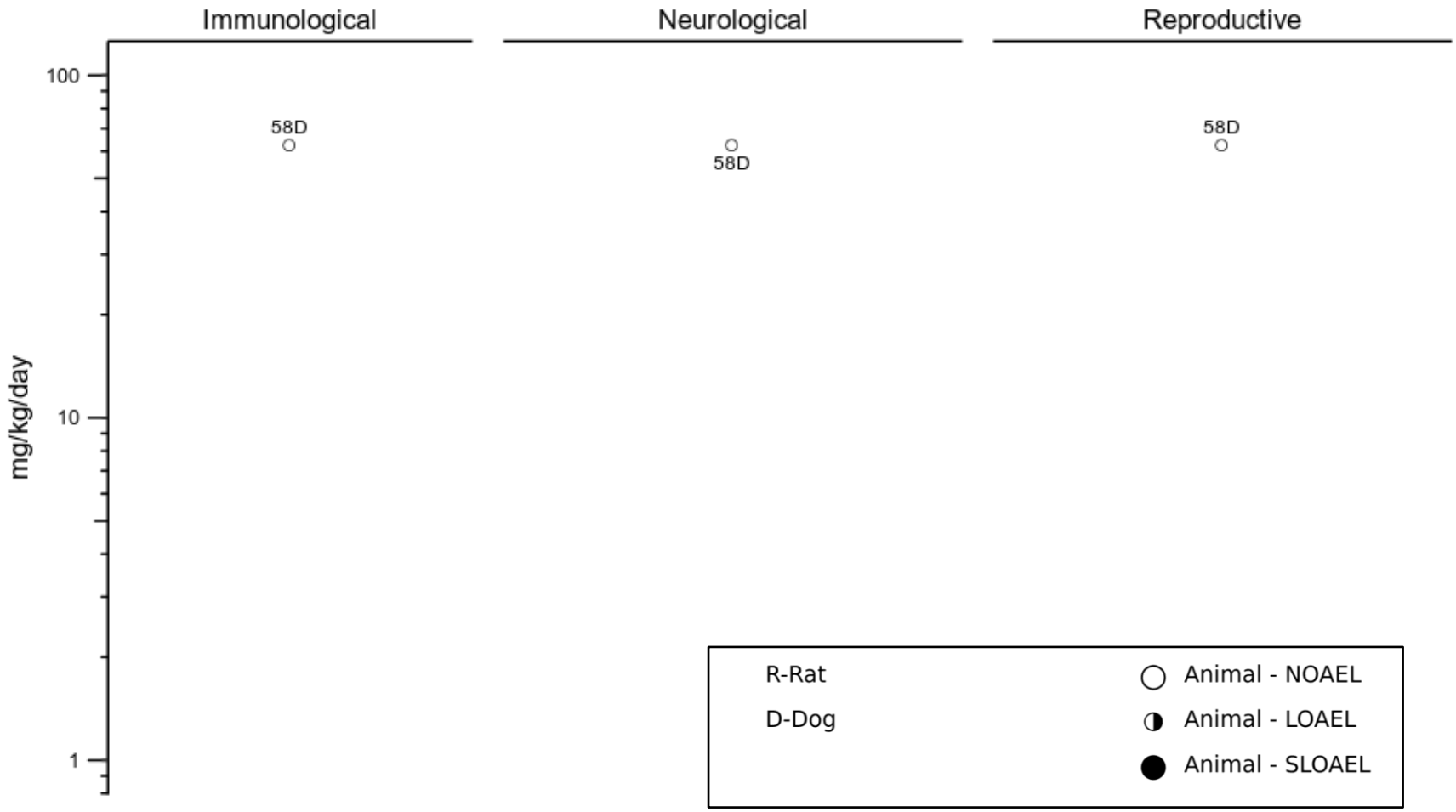

Figure 2-34. Levels of Significant Exposure to Nickel – Oral
Chronic (≥365 days)

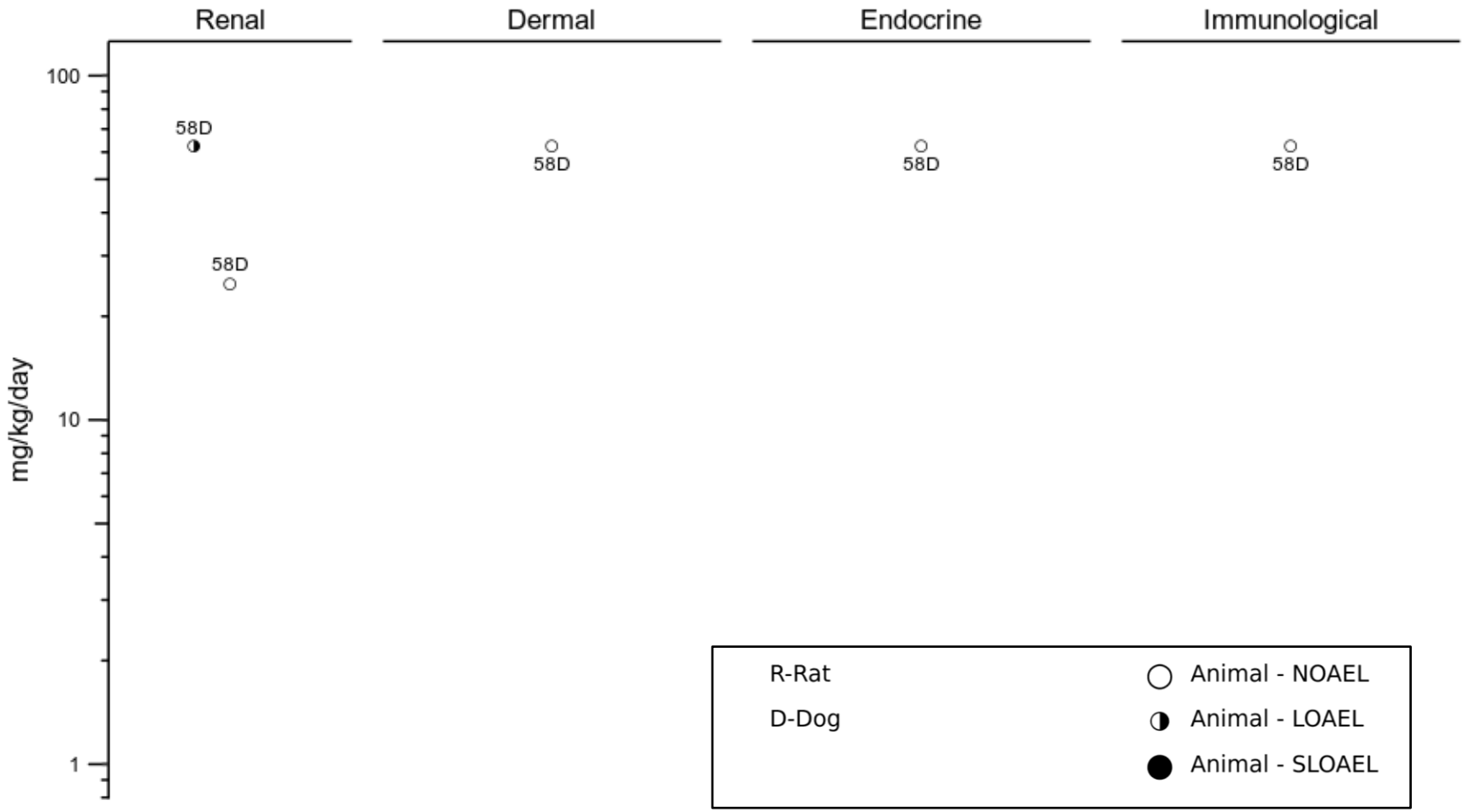

Figure 2-35. Levels of Significant Exposure to Nickel – Oral
Chronic (≥365 days)

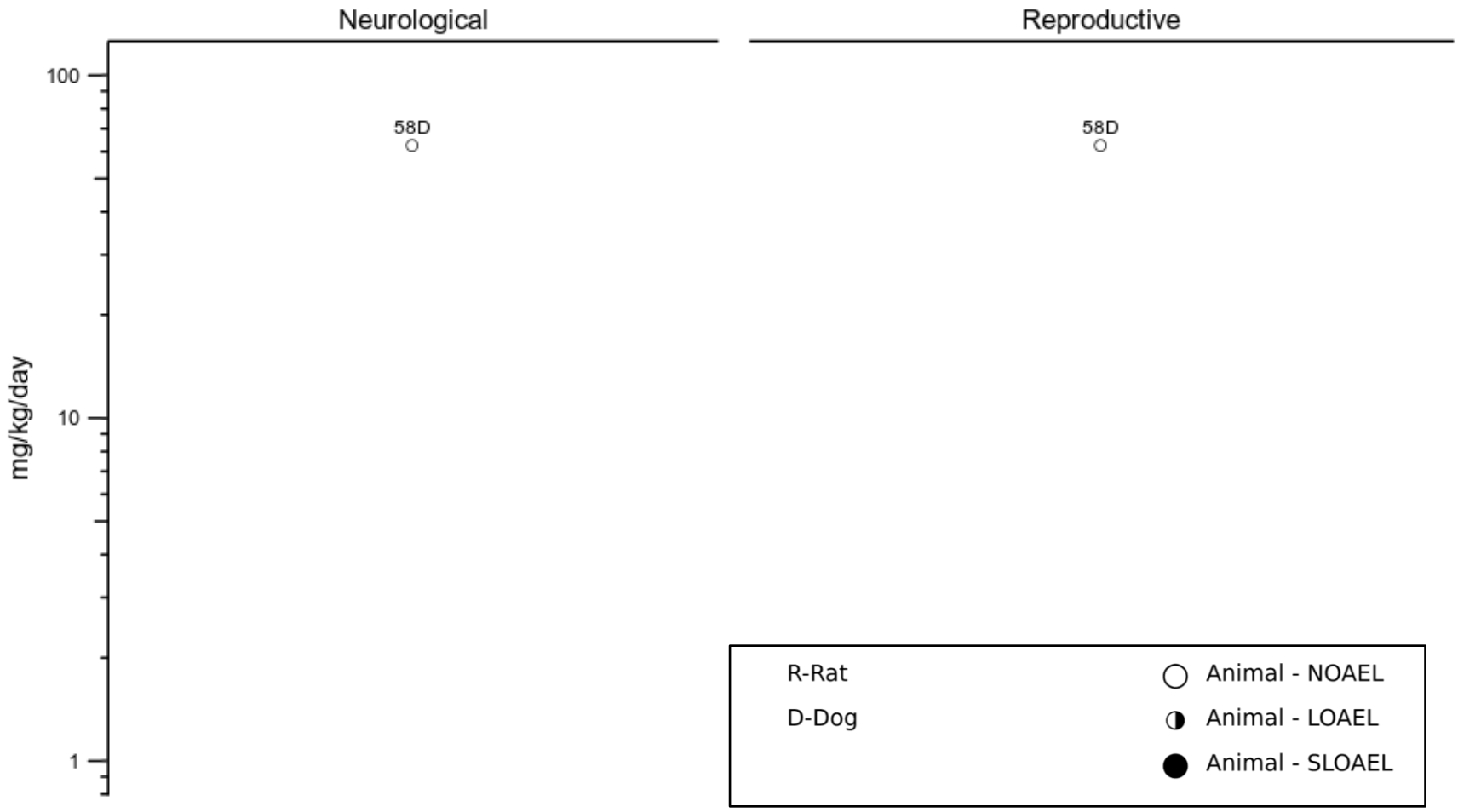

Table 2-3. Levels of Significant Exposure to Nickel – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE								
Emmett et al. 1988								**Nickel sulfate**
HUMAN 12 NS	Once	0–47 mg (0.01%) - 5.2 mg (2.5%)	CS	Dermal	0.01%	0.03%		Contact dermatitis in sensitive individuals
Eun and Marks 1990								**Nickel sulfate**
HUMAN 20 NS	Once	0.04 - 5%	CS	Dermal		0.04%		Allergic dermatitis in sensitive individuals
Menne and Calvin 1993								**Nickel chloride**
HUMAN 16-51 NS	Once	0, 0.1, 1, 10, 100, 1000, 4000 ppm	CS	Dermal	0.01 ppm	0.1 ppm		Skin reaction in nickel sensitive individuals
Menne et al. 1987								**Nickel alloys**
HUMAN 164F 9M	Once	1 mg/cm^2/week	CS	Dermal		1 mg/cm^2/week		Contact dermatitis
Siller and Seymour 1994								**Nickel sulfate**
MOUSE (C3H:Hej) 4F	once for 7 days	0, 1, 5, 10, 15, 20%	CS	Immuno		1%		Development of dermal sensitization
INTERMEDIATE EXPOSURE								
Mathur et al. 1977								**Nickel sulfate**
RAT (NS) 8M	15 or 30 days daily	0, 40, 60, 100 mg/kg	CS GN HP RX	Hepatic	40 mg/kg	60 mg/kg		Focal necrosis
				Renal	100 mg/kg			
				Dermal		40 mg/kg		Slight hyperkeratosis
				Repro	40 mg/kg		60 mg/kg	Degeneration and edema of seminiferous tubules

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

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Mathur and Gupta 1994								**Nickel sulfate**
GN PIG (NS) 12NS	15 or 30 days	0, 100 mg/kg	BC	Hemato	100 mg/kg			
				Hepatic		100 mg/kg		Increased Mg2+ ATPase, acid phosphatase, and glucose-6-phosphatase activities
				Renal		100 mg/kg		Increased Mg2+ ATPase activity
				Other noncancer		100 mg/kg		Increased blood glucose

ATP = adenosine triphosphate; CS = clinical signs; F= female(s); HE = hematological; Immuno = immunological; LOAEL = lowest-observed-adverse-effect-level; M = male(s); NOAEL = no-observed-adverse-effect-level; NS = not specified; Repro = Reproductive; RX = reproductive function; SLOAEL = serious lowest-observed-adverse-effect-level

2.2 DEATH

Inhalation

Death from ARDS was reported in one person who sprayed nickel with a metal arc process without wearing personal protective equipment (Rendall et al. 1994). Death occurred 13 days after a 90-minute exposure to an estimated concentration of 382 mg Ni/m^3 of principally metallic nickel with the majority of particle sizes of <1.4 µm (Sunderman 1993). Histological examination of the lungs revealed alveolar wall damage and edema in alveolar spaces, and marked tubular necrosis was noted in the kidneys. A case-series detailing 7 workers of a waste-processing factory who were admitted to the hospital following nickel carbonyl poisoning reported 3 deaths with autopsies revealing interstitial lung fibrosis (Seet et al. 2005). In a fatal case of an adult male worker exposed to nickel carbonyl vapor for an estimated 30 minutes to several hours, imaging showed pneumonitis following presentation with dyspnea and hypoxia (Rusin et al. 2019).

Human data regarding chronic-duration inhalation exposure to nickel are limited to occupational exposure studies. Most of these studies analyzed the toxicity of nickel, usually in the form of nickel oxide, metallic nickel, or nickel refinery dust, by calculating Standard Mortality Ratios (SMR) for all causes of death. Generally, the studies report a higher incidence of cancer deaths from lung and nasal cancers in the exposed workers (see Section 2.19 Cancer). Two studies have also reported a higher incidence of deaths resulting from nonmalignant respiratory disease (Cornell and Landis 1984; Polednak 1981). However, all of the workers were exposed to other metals (arsenic, uranium, iron, lead, chromium) and non-metallic substances, so it cannot be concluded that nickel was the sole causative agent. Other studies of humans occupationally exposed to nickel compounds have not reported increased mortality resulting from respiratory diseases (Cox et al. 1981; Cragle et al. 1984; Enterline and Marsh 1982; Redmond 1984; Shannon et al. 1984b; Shannon et al. 1991).

During the first 2 days after a single 2-hour exposure, 4 out of 28 Fischer-344 rats died after exposure to nickel sulfate at 36.5 mg Ni/m^3 (Hirano et al. 1994). Severe hemorrhage of the lungs was observed in the lungs of the rats that died. Significant mortality was observed during the last 26 weeks of a 31-month inhalation study of Fischer-344 rats exposed to 0.63 mg Ni/m^3 as nickel sulfide (Ottolenghi et al. 1975). Less than 5% of the treated rats survived the study (78 weeks of exposure plus 30 weeks of observation) compared to 31% of the controls (Ottolenghi et al. 1975). A significant decrease in mean survival time was observed in Wistar rats exposed 23 hours/day for life to 0.06 mg Ni/m^3 as nickel oxide (Takenaka et al. 1985). Male and female Wistar rats showed reduced survival by 72% and 48% respectively by 103 weeks of continuous exposure (5 days/week, 6 hours/day) (Oller et al. 2008)

NTP studies observed that B6C3F1 mice were more sensitive to lethality from nickel exposure than Fischer-344 rats. At 1.4 mg Ni/m^3 as nickel sulfate hexahydrate, all mice and no rats died, and at 7.33 mg Ni/m^3 as nickel subsulfide, all mice and only 2 of 10 rats died following exposure for 6 hours/day, 5 days/week, for up to 12 exposures (NTP 1996a, 1996b, 1996c). No rats or mice died following exposure to 23.6 mg Ni/m^3 as nickel oxide. No deaths were reported in rats or mice following 13 weeks of exposure (6 hours/day, 5 days/week) to nickel at 7.9, 1.83, or 0.44 mg Ni/m^3 as nickel oxide, nickel subsulfide, or nickel sulfate, respectively (NTP 1996a, 1996b, 1996c). The average survival times for rats exposed to 0 or 0.06 mg Ni/m^3 were 125.2 and 87.7 weeks, respectively. Survival was not affected in rats exposed to nickel oxide, nickel subsulfide, or nickel sulfate at concentrations up to 2, 0.73, or 0.11 mg Ni/m^3, respectively, for 104 weeks (NTP 1996a, 1996b, 1996c). Survival of mice was also not affected by exposure to nickel oxide, nickel subsulfide, or nickel sulfate at concentrations up to 3.9, 0.88, or 0.22 mg Ni/m^3, respectively, for 104 weeks (NTP 1996a, 1996b, 1996c).

All rats (Bethesda Black), guinea pigs (Strain 13), and mice (C57) exposed to 15 mg Ni/m^3 as metallic nickel for 21 months died before the end of the study, with most of the guinea pigs and mice dying by 15 months (Hueper 1958). Lung lesions including edema, hyperemia, and hemorrhage were the principal causes noted. A major study deficiency was the lack of control animals, the study instead compared exposure groups to data of same-species controls from previous carcinogenic studies (Hueper 1958).

Oral

One human death following oral exposure to nickel was reported (Daldrup et al. 1983). A 2-year-old child accidentally ingested nickel sulfate crystals (rough estimate of 570 mg Ni/kg). Four hours after ingestion, cardiac arrest occurred, and the child died 8 hours after exposure.

Oral LD_{50} values of 116 and 136 mg Ni/kg as nickel acetate in Fischer-344 female rats and male Swiss-albino mice, respectively have been reported for soluble nickel compounds (Haro et al. 1968). Single-dose oral lethality studies indicate that soluble nickel compounds are more toxic than less-soluble nickel compounds.

Increases in mortality (6/52, 60/60) were observed in Sprague-Dawley rats administered via gavage 8.6 or 25 mg Ni/kg/day as nickel chloride hexahydrate for 91 days (American Biogenics Corporation 1988). Clinical signs observed included lethargy, ataxia, irregular breathing, hypothermia, salivation, squinting, and loose stools. As part of a longer-term study, Sprague-Dawley rats were provided with drinking water containing 1,000 ppm nickel as nickel chloride (approximately 140 mg/kg/day) (RTI 1988a). Within 2 weeks, 7/62 died and the dose was eliminated from the study. Over a 2-year study, mortality in female Fischer-344 rats exposed to 2.232 mg Ni/m^3 as nickel sulfate hexahydrate was 33% and the increase with

dose was an exposure-response to nickel (Heim et al. 2007). No exposure-related response was seen in male rats exposed during the same period. In other studies, no deaths were observed in Sprague-Dawley rats given 28.8 mg Ni/kg/day as nickel sulfate in drinking water for 13 weeks (Obone et al. 1999), or Fischer-344 rats given 22 mg Ni/kg/day (males) or 33 mg Ni/kg/day (females) as nickel sulfide administered via gavage for 90 days (Springborn Laboratories 2002); no deaths were observed in B6C3F1 mice provided with nickel sulfate in the drinking water at doses up to 150 mg Ni/kg/day for 180 days (Dieter et al. 1988).

In a multigeneration study (RTI 1988a, 1988b) in which CD rats were treated with nickel chloride in the drinking water, the death of female rats from pregnancy complications at the time of delivery suggests that females are more susceptible to nickel toxicity during parturition. Although the number of deaths was not significantly above controls and not clearly dose related (P0: 0/31 in controls, 1/31 at 7 mg/kg/day, 3/30 at 30 mg/kg/day, and 3/31 at 55 mg/kg/day; F1: 0/30 at 0 and 7 mg/kg/day, 3/30 at 30 mg/kg/day, and 1/30 at 55 mg/kg/day), death in dams during delivery is a relatively rare event. The results of this study (RTI 1988a, 1988b) are confounded by a decrease in food and water intake observed in the exposed animals. Deaths in offspring before weaning have also been reported in multigeneration, multi-littered studies (RTI 1988a, 1988b; Schroeder and Mitchener 1971; Smith et al. 1993). Because cross-fostering studies have not been completed, it is not possible to know if the pre-weaning deaths are a result of an inherent defect in the pups, nickel exposure through the milk, or a change in the quality or quantity of the milk produced by the dam (Smith et al. 1993).

An increase in mortality was not observed in chronic-duration studies in Wistar rats or Beagle dogs fed nickel sulfate in the diet at doses up to 188 mg/kg/day for rats and 62.5 mg/kg/day for dogs (Ambrose et al. 1976).

Dermal

No studies were identified that examined death in humans or animals after dermal exposure to nickel.

2.3 BODY WEIGHT

Inhalation

No studies were located regarding body weight effects in humans after inhalation exposure to nickel.

No exposure-related body weight changes are observed in female ICR mice exposed 24-hours whole body to concentrations up to 0.0801 mg Ni/m^3 as nickel chloride hexahydrate (Buxton et al. 2021). Acute continuous exposure of 23.6 mg Ni/m^3 as nickel oxide for 12 days in a 16-day period did not affect body weight in Fischer-344 rats and B6C3F1 mice of both sexes (NTP 1996a). Subsequent studies from the

National Toxicology program observed significant decreases in body weight (22-28%) of Fischer-344 rats after 12 days of continuous exposure to 0.7 to 1.83 mg Ni/m^3 nickel sulfate hexahydrate and nickel subsulfide (NTP 1996b, 1996c). Male and female B6C3F1 mice exposed for a similar duration to 1.4 mg Ni/m^3 of nickel sulfate appeared emaciated (NTP 1996c) while a similar study observed male mice final body weight from exposure to 3.65 mg Ni/m^3 of nickel subsulfide was 14% less than controls (NTP 1996b). Based on these NTP studies, body weight changes appear to be sensitive to an acute low dose exposure to nickel (NTP 1996a, 1996b, 1996c). Acute-duration exposure in Fischer-344 rats to nickel subsulfide at concentrations of 0.3 to 0.43 mg Ni/m^3 for 1 week (5 days/week, 6 hours/day) did not result in any body weight changes (Efremenko et al. 2014). When Fischer-344 rats of both sexes were exposed to 1.83 mg Ni/m^3 of metallic nickel, body weight decreased by 17-19 % after 7 days of exposure (Benson et al. 1995b).

Intermediate-duration continuous exposure of 7.9, 1.83, 0.44 mg Ni/m^3 for 13 weeks (5 days/week, 6 hours/day) did not affect body weight in Fischer- 344 rats and B6C3F1 mice of both sexes (NTP 1996a, 1996b, 1996c). No exposure-related body weight changes were seen in male Fischer-344 rats exposed continuously to 1.96 mg Ni/m^3 metallic nickel for 2-6 months (Benson et al. 1995a) and exposed to 0.03 to 0.45 mg Ni/m^3 as nickel subsulfide for 4 weeks, 5 days/week, 6 hours/day (Efremenko et al. 2014). Similarly, no effect on body weight was reported in Long-Evans rats exposed to 0.635 mg Ni/m^3 nickel sulfate hexahydrate for 16 days (Evans et al. 1995). Conversely, other studies in rats have observed exposure-related body weight changes at concentrations ranging from 0.385 to 1.83 mg Ni/m^3 as nickel oxide, nickel chloride, or nickel subsulfide (Benson et al. 1995b; Weischer et al. 1980). Male and female Fischer-344 rats showed a 10-19% decrease in body weight follow exposure to 1.83 mg Ni/m^3 for 22 days, 6 hours/day (Benson et al. 1995b). Weischer et al. (1980) reported 30–36% decreases in body weight gain in male and female Wistar rats exposed to 0.385 or 0.8 mg Ni/m^3, respectively, continuously for 21–28 days. In pregnant rats, an 11% decrease in body weight gain was observed at 0.8 mg Ni/m^3 compared to the 36% decrease observed in similarly exposed non-pregnant rats (Weischer et al. 1980).

Two intermediate-duration studies in mice did not find exposure related changes in body weight (Benson et al. 1995a; Xu et al. 2012). Neither exposure to 0.00017 mg Ni/m^3 for 3 months, 5 days/week, 6 hours/day in ApoE-/- mice (Xu et al. 2012) nor continuous exposure to 3.9 mg Ni/m^3 in B6C3F1 mice for 2-6 months resulted in body weight changes (Benson et al. 1995a).

Chronic-duration continuous exposure of 2 mg Ni/m^3 for 2 years (5 days/week, 6 hours/day) did not affect body weight in Fischer- 344 rats of both sexes (NTP 1996a). Under identical exposure conditions a concentration of 3.9 mg Ni/m^3 as metallic nickel did not change body weight in B6C3F1 mice of both sexes (NTP 1996a). In NTP (1996b), chronic-duration exposure to metallic nickel at 0.73 mg Ni/m^3

resulted in a 11-12% decrease in body weight in Fischer-344 rats of both sexes but had no effect in B6C3F1 mice at a concentration of 0.88 mg Ni/m^3. In NTP (1996c), chronic-duration continuous (5 days/week, 6 hours/day) exposure to nickel sulfate hexahydrate at 0.22 mg Ni//m^3 decreased body weight by 12% in female B6C3F1 mice but not in male mice. Similar duration of exposure at 0.11 mg Ni/m^3 had no effect on body weight in Fischer-344 rats of both sexes (NTP 1996c). Ottolenghi et al. (1975) observed a 20-30% decrease in body weight in male and female Fischer-344 rats compared to controls after exposure to metallic nickel at 0.63 mg Ni/m^3. Chronic-duration exposure to metallic nickel at 0.06 to 0.942 mg Ni/m^3 in male Wistar rats did not affect body weight (Takenaka et al. 1985; Tanaka et al. 1988).

Oral

No studies were identified that examined body weight effects in humans after oral exposure to nickel.

A dose-dependent reduction in body weight gain was observed in treated animals compared to the control group. This reduction of body weight gain was associated with reduced food and/or water intake reported in Wistar rats orally exposed to 0.23 to 0.97 mg Ni/kg/day as nickel chloride in drinking water for 28 days (Weischer et al. 1980); in Sprague-Dawley rats treated by gavage with 8.6 mg Ni/kg/day as nickel chloride for 91 days (American Biogenics Corporation 1988) or 55 mg Ni/kg/day for 30 weeks (RTI 1988a); and in Wistar rats treated with 75 mg Ni/kg/day of nickel sulfate hexahydrate for 2 years in the diet (Ambrose et al. 1976). The concomitant decreases in food and/or water consumption limit the interpretation of these results. Decreases (10–13%) in body weight gain were also observed in male and female Fischer-344 rats administered via gavage 17 or 28 mg Ni/kg/day, respectively, as nickel sulfate (Springborn Laboratories 2002); however the decreases in body weight gain were not associated with consistent alterations in food intake (water consumption data were not reported). Male and female Fischer-455 rats exposed to 6.69 and 11.16 mg Ni/kg/day as nickel sulfate hexahydrate, respectively, for 2 years daily showed an average body weight decrease of 10-11% compared to controls (Heim et al. 2007). In the 90-day intermediate-duration study by Heim et al. (2007) similar body weight decreases were reported in rats when males and females were exposed to 16.74 and 27.91 mg Ni/kg/day, respectively. In brown rats, no body weight changes were reported following a 6-week exposure to 5 mg Ni/kg/day as nickel acetate in feed (Whanger 1973). However, body weight gain was significantly decreased by 88% compared to controls at doses ≥25 mg Ni/kg/day.

Decreases in body weight gain of 10% or more were not observed in various studies in rats, including Sprague-Dawley rats exposed to nickel sulfate in drinking water at 28.8 mg Ni/kg/day for 13 weeks (Obone et al. 1999), in Wistar rats exposed by gavage at 7.58 mg Ni/kg/day for 21 days (Adeyemi et al. 2017), or in Sprague-Dawley rats by gavage at up to 2.2 mg Ni/kg/day for 18 weeks daily (Springborn

Laboratories 2000a). Similarly, no exposure related effects were reported in rats treated with nickel chloride in drinking water at 31.6 mg Ni/kg/day for 11 weeks (Smith et al. 1993), nickel sulfate in drinking water at 28.8 mg Ni/kg/day for 13 weeks (Obone et al. 1999), or nickel sulfate at a dose of 7.6 mg Ni/kg/day for 3 or 6 months (Vyskocil et al. 1994b).

Decreased body weight gain has also been reported in mice treated with nickel chloride in feed at 4.53 mg Ni/kg/day for 3-12 weeks daily (Toman et al. 2012), nickel sulfate in drinking water at a dose of 108 mg Ni/kg/day for 180 days (Dieter et al. 1988), and in dogs treated with nickel sulfate in the diet at a dose of 62.4 mg/kg/day for 2 years (Ambrose et al. 1976). Female ICR mice treated with 90.6 mg Ni/kg/day as nickel chloride during gestation days 8-12 showed weight gain 49% lower than controls (Seidenberg et al. 1986). Male BALB/c mice exposed to doses ranging from 0.9 to 7.2 mg Ni/kg/day as nickel chloride did not show any exposure-related changes in body weight (Gathwan et al. 2013).

Dermal

No studies were identified that examined body weight in humans or animals after dermal exposure to nickel.

2.4 RESPIRATORY

Inhalation

Numerous human studies have examined the potential of nickel and nickel compounds to induce respiratory effects. Most of these studies were cohort mortality studies in nickel exposed workers. A significant excess of deaths from nonmalignant respiratory system disease was found among foundry workers that was associated with the duration of foundry employment, regardless of exposure to nickel (Cornell and Landis 1984). Other studies of refinery workers or workers exposed to nickel alloys have not found increases in deaths from respiratory disease (Arena et al. 1998; Cox et al. 1981; Cragle et al. 1984; Egedahl et al. 2001; Enterline and Marsh 1982; Redmond 1984; Roberts et al. 1989a; Shannon et al. 1984b; Shannon et al. 1991). Two studies of welders also did not find significant increases in the risk of nonmalignant respiratory disease deaths (Moulin et al. 2000; Polednak 1981). A common limitation of the cohort mortality studies is that the number of observed deaths from all causes were lower (in many cases significantly lower) than the number expected deaths, suggesting a healthy worker effect. Additionally, the workers were exposed to other respiratory toxicants; this is particularly true for welders exposed to elevated levels of chromium. A single case of death from ARDS has been reported following a 90-minute exposure to a very high concentration (382 mg/m^3) of metallic nickel of small particle size (<1.4 µm)

(Rendall et al. 1994). Histological changes noted in the lungs of this case included alveolar wall damage, with fibrotic changes, and edema in the alveolar space.

A small number of studies have examined potential respiratory tract effects, not associated with lethality. An industrial hygiene survey of welders in New Zealand reported a significant odds ratio for workers currently exposed to high nickel levels (0.001-0.002 mg/m^3) and work-related respiratory symptoms (adjusted OR=7.0, 1.3-36.6) (Fishwick et al. 2004). Study authors reported that detailed exposure information was not available however exposure to welding fumes considered workplace factors, respiratory protection, and ventilation (Fishwick et al. 2004). Reduced vital capacity and expiratory flows were observed in stainless steel welders exposed to elevated levels of nickel and chromium (Kilburn et al. 1990). Ninety welders were selected to participate in the study and results were compared against the predicted values obtained through regression analysis of a random population of men (reference population). Welders did not wear respiratory protection nor were local area ventilation devices used. When results in welders were stratified based on smoking status, among non-smokers, only the forced expiratory volume (FEV_{75-85}) was significantly different from the predicted measurement based on the reference population. Thus, suggesting that current smoking status may have contributed to the observed effects. The study also found that the prevalence of chronic bronchitis was higher among all exposed welders regardless of smoking status when compared to predicted values from the reference population. Although this study provides suggestive evidence of respiratory effects in welders, establishing a causal relationship between nickel and the observed effects is limited by co-exposure to chromium. Additional limitations include use of predicted population values based on a random sample of men as the comparison group, rather than a comparison group of non-nickel-exposed welders. Examination of chest radiographs of nickel sinter plant workers exposed to nickel while wearing protective masks at concentrations as high as 100 mg/m^3 did not reveal an increase in small irregular opacities, which would be indicative of an inflammatory or fibrogenic response in the lungs (Muir et al. 1993). Another study, which did not state if personal protective equipment was used, found an increased risk of moderate pulmonary fibrosis, after controlling for age and smoking, among nickel refinery workers with cumulative exposure to soluble nickel or sulfidic nickel (Berge and Skyberg 2003). A dose-response trend was also found for soluble nickel among cases in the three highest cumulative exposure categories (0.04–≤0.15, 0.15–≤0.6, and >0.6 mg/m^3 x years), after adjusting for age, smoking, and exposure to asbestos. Asthma induced by occupational exposure to nickel has been documented in a small number of individuals (Dolovich et al. 1984; Novey et al. 1983; Shirakawa et al. 1990). Asthma can result from either primary irritation or an allergic response. Interpretation of these data is limited by the small number of cases, as well as by possible exposure to other sensitizing metals.

Several case studies of workers exposed to nickel corroborate the respiratory system as a sensitive endpoint of inhalation exposure. A 55-year-old male who had cleaned a nickel carbonyl reaction vessel had sought medical care 2 days after exposure and imaging showed pneumonitis following presentation with dyspnea and hypoxia (Rusin et al. 2019). The worker died 44 days after exposure and had also developed diarrhea, acute kidney injury, and leukocytosis during treatments; an investigation by OSHA indicated that the worker had likely inhaled nickel carbonyl vapor for 30 minutes to several hours (Rusin et al. 2019). Nausea, myalgia, and cough was reported by a 50-year-old industrial worker who presented to the hospital 12-24 hours after exposure to an unknown concentration of nickel carbonyl (Bowman et al. 2018). Additional testing revealed that forced expiratory volume (FEV1) and forced vital capacity (FVC) were lower than predicted. The patient's urine nickel level on admission was 692 µg/L (reference value: <10 mcg/L) (Bowman et al. 2018). Lung injury was seen in a 50-year-old welder who accidentally inhaled an unknown concentration of nickel fumes that was being sprayed while not wearing any personal protective equipment (Kunimasa et al. 2011). The patient immediately developed a persistent strong cough and a chest radiograph three days later showed reticular opacities in middle and lower lung fields, while a CT scan of the chest showed bilateral non-segmental ground-glass opacities. A 29-year-old metallic coating and nickel-plating worker, exposed for 5 years, presented with nasal septal perforation; exposure was further indicated by elevated nickel concentrations in serum and urine samples (Bolek et al. 2017). A 27-year-old male metalworker presented with nasal obstruction and mild right-sided epistaxis and reported 6 years of exposure to a dry furnace dust of "nickel matte" (50% nickel, 30% copper, 20% sulfur and trace amounts of other metals) (Peric and Durdevic 2020). Histological examination of a lesion in the paranasal sinuses showed an inflammatory nasal polyp.

Several population studies have also examined associations of nickel in ambient air and various respiratory system effects. Two studies specifically looked at respiratory and cardiovascular hospitalizations in adults over 65 years old and found an association with higher nickel in $PM_{2.5}$ (Bell et al. 2009; Bell et al. 2014). Bell et al. (2009) looked at hospitalizations in 106 U.S. counties from 1999 to 2005, while Bell et al. (2014) analyzed 4 counties in the Northeast from 2000 to 2004.

Several other studies have examined respiratory effects in children. Increases in ambient air nickel concentrations were significantly associated with increased probability of wheeze among a cohort of children up to 24 months of age living in New York City between 1998 and 2006 (Patel et al. 2009). In a separate prospective case-control study of thirty-six 6-to-14 year old children in New York City, nickel in air was significantly associated with maximum asthma symptoms including cough and wheeze in the winter; odds ratio of 1.94 (1.08-3.49) (Schachter et al. 2020). Additionally, increased albuterol use (asthma inhaler) was significantly associated with nickel (odds ratio=2.27; 1.02-5.07), however this effect

disappeared when adjusted for ozone. In a single pollutant model, reports of asthma in children 11-14 years of age were associated with nickel exposure, as a relative risk of 1.11 was calculated per 4 ng/m^3 increase (Rosa et al. 2016). A prospective birth cohort which followed children from birth up 12 years of age found no associations between Ni $PM_{2.5}$ or Ni PM_{10} and parent-reported asthma symptoms or incidents (Gehring et al. 2015).

Studies in rats and mice demonstrate that chronic active inflammation in the lungs is the most prominent effect following inhalation exposure to nickel sulfate, nickel subsulfide, or nickel oxide. In acutely exposed Fischer-344 rats, chronic lung inflammation was observed at the lowest nickel sulfate (0.7 mg Ni/m^3) and nickel subsulfide (0.44 mg Ni/m^3) concentrations tested in 12-day exposure studies (6 hours/day, 12 days in a 16-day period) (NTP 1996b, 1996c). At higher concentrations of nickel sulfate and nickel subsulfide (1.4 and 3.65 mg Ni/m^3, respectively), the inflammation was accompanied by labored breathing. The chronic active lung inflammation was characterized by focal accumulation of alveolar macrophages and interstitial (nickel subsulfide) or inflammatory cell (nickel sulfate) infiltrates. At the higher concentrations, necrotic cellular debris were also present. Bronchiolar epithelium degeneration was also observed in rats exposed to 0.7 mg Ni/m^3 as nickel sulfate (NTP 1996c). Consistent with these findings, is the observation of alveolitis in Fischer-344 rats exposed to 0.44 mg Ni/m^3 as nickel subsulfide 6 hours/day for 7 days (Benson et al. 1995b). Additionally, exposure to 1.83 mg Ni/m^3 as nickel subsulfide resulted in alveolitis and alveolar proteinosis after 4 days of exposure (Benson et al. 1995b). In contrast, acute lung inflammation, consisting of neutrophilic infiltrates, was first observed in rats exposed to nickel oxide at 7.9 mg Ni/m^3 (NTP 1996a); chronic lung inflammation was not observed at doses as high as 23.6 mg Ni/m^3. Mice appear to be less sensitive than rats to the acute toxicity of nickel with LOAELs for chronic inflammation of 0.7, 1.83, and >23.6 mg Ni/m^3 as nickel sulfate, nickel subsulfide, and nickel oxide, respectively (NTP 1996a, 1996b, 1996c). Bai et al. (2013) exposed Sprague-Dawley rats to concentrations of 6.88, 46.47, and 85.94 mg Ni/m^3 as nickel carbonyl for 30 minutes in an inhalation chamber and damage of type II alveolar epithelial cells was apparent in rat lung tissue of all exposure groups. A dose-effect relationship was indicated based on the increasing severity of damage. The highest exposure group showed pulmonary tissue edema and decreased peroxidation of pulmonary tissue (Bai et al. 2013). Lung histopathology in 5 out of 5 Fischer-344 rats exposed to 0.43 mg Ni/m^3 as nickel subsulfide showed peribronchiolar/perivascular inflammation following 1 week of exposure (5 days/weeks for 6 hours/day) (Efremenko et al. 2014). Inflammation was characterized by “peribronchiolar and perivascular edema, lymphocytes and occasional neutrophils.” When exposed for 20 days over 4 weeks, 5 out of 5 rats exposed to 0.11 mg Ni/m^3 had minimal to mild alveolar inflammation. No effects were seen at 4 weeks of exposure to concentrations ≤0.06 mg Ni/m^3 (Efremenko et al. 2014).

As with acute-duration exposure, chronic lung inflammation was typically observed at the lowest adverse effect level following intermediate-duration exposure. Thirteen-week (6 hours/day, 5 days/week) NTP studies of rats exposed to nickel sulfate, nickel subsulfide, or nickel oxide (NTP 1996a, 1996b, 1996c) identified LOAELs for chronic active lung inflammation of 0.11, 0.22, and 3.9 mg Ni/m^3, respectively; NOAEL values of 0.06, 0.11, and 2 mg Ni/m^3, respectively, were also identified for chronic inflammation.

Oller et al. (2022) reported increased incidence of alveolitis, proteinosis, and perivascular/peribronchiolar inflammation in Fischer-344 rats exposed to 0.04 mg Ni/m^3 as nickel subsulfide for 13 weeks (6 hours/day, 5 days/week). The incidence and severity of lung lesions at 3 and 13 weeks of exposure showed that increases in both are concentration dependent. Rats exposed under similar conditions to nickel sulfate hexahydrate showed similar concentration-dependent results in pulmonary lesions (Oller et al. 2022). At a NOAEL of 0.03 mg Ni/m^3 as nickel sulfate hexahydrate, there was no difference between the exposed rats and controls for incidence of lung inflammation or lesions, or changes in lung weight. At 0.11 mg Ni/m^3 as nickel sulfate hexahydrate, the incidence of alveolitis, perivascular/peribronchiolar inflammation, and bronchiolar epithelial degeneration and apoptosis was high. In addition, increases in LDH levels in bronchoalveolar lavage fluid (BALF) were significant at 0.11 mg Ni/m^3 as nickel sulfate hexahydrate (Oller et al. 2022). Comparison of lesions showed that the incidence and severity of perivascular/peribronchiolar lesions and alveolar type II cell hyperplasia was higher in rats exposed to nickel subsulfide (Oller et al. 2022). Alveolitis was reported in rats exposed to 0.11 mg Ni/m^3 as nickel sulfate and 1.96 mg Ni/m^3 as nickel oxide for 6 months (6 hours/day, 5 days/week) (Benson et al. 1995a). Similarly, localized interstitial pneumonia, represented by lymphoid infiltration and fibrosis of alveolar septa, emphysema, and atelectasis of varying degree, was seen in rats exposed to 0.5 mg Ni/m^3 as nickel oxide for 1 month (Horie et al. 1985). In the study by Oller et al. (2022), one group of rats was exposed to a high dose of nickel sulfate hexahydrate (0.44 mg Ni/m^3) but died within the first week of exposure, and the deaths were attributed to respiratory toxicity. Rats showed labored breathing and nasal discharge; gross necropsy showed severe pulmonary edema as the likely cause of death (Oller et al. 2022).

Several other lung effects have also been observed in rats exposed to nickel for intermediate durations. Minimal alveolar macrophage hyperplasia was observed at the lowest nickel sulfate, nickel subsulfide, and nickel oxide concentrations evaluated (0.03, 0.11, and 0.4 mg Ni/m^3, respectively) (NTP 1996a, 1996b, 1996c). These slight changes in the number of macrophages were not considered adverse because it is considered part of the normal physiologic response to inhaled particles, and it is not believed to compromise the lung's ability to clear foreign matter. This is supported by results from Oller et al. (2022) where the incidence of alveolar macrophage hyperplasia was similar between controls and groups of rats

exposed to concentrations of nickel sulfate hexahydrate or nickel subsulfide up to 0.22 and 0.44 mg Ni/m^3, respectively. However, the increased severity of this lesion appears to be concentration related (Oller et al. 2022). At higher nickel concentrations, mild to moderate changes in alveolar macrophage hyperplasia were found. Interstitial infiltrates were observed in rats exposed to ≥0.11 or 0.22 mg Ni/m^3 as nickel sulfate or nickel subsulfide (NTP 1996b, 1996c) or 0.109 mg Ni/m^3 as nickel chloride (Bingham et al. 1972), granulomatous inflammation was observed in rats exposed to 3.9 mg Ni/m^3 as nickel oxide (NTP 1996a), alveolar wall thickening was observed in rats exposed to 0.12 mg Ni/m^3 as nickel oxide (Bingham et al. 1972), and hyperplasia of the bronchial epithelium was observed in rats exposed to 0.109 mg Ni/m^3 as nickel chloride (Bingham et al. 1972). The highest NOAEL values for respiratory effects in rats exposed to nickel sulfate, nickel subsulfide, or nickel oxide for intermediate-durations were 0.06 mg Ni/m^3 (NTP 1996c), 0.11 mg Ni/m^3 (NTP 1996b), and 0.49 mg Ni/m^3, respectively (Benson et al. 1995a). An intermediate-duration inhalation MRL was derived from the NOAEL (0.06 mg Ni/m^3) and LOAEL (0.11 mg Ni/m^3) identified from the NTP (1996c) study of nickel sulfate.

Similar effects have been observed in mice exposed to nickel for intermediate durations, although the LOAELs for the lung effects tend to be higher suggesting a lower sensitivity compared to rats. Chronic active lung inflammation was observed in mice exposed to ≥0.44 and 0.88 mg Ni/m^3 as nickel sulfate or nickel subsulfide, respectively (NTP 1996b, 1996c). Lung inflammation was not found in mice exposed to nickel oxide at concentrations as high as 7.9 mg Ni/m^3 (NTP 1996a); however, perivascular lymphocyte infiltrates were observed at 3.9 and 7.9 mg Ni/m^3 (NTP 1996a). Interstitial pneumonia has also been observed in mice exposed to 0.22 or 0.98 mg Ni/m^3 as nickel sulfate or nickel oxide (Benson et al. 1995a). Other lung effects in mice include minimal alveolar macrophage hyperplasia at 0.11, 0.22, or 0.4 mg Ni/m^3 as nickel sulfate, nickel subsulfide, or nickel oxide, respectively (NTP 1996a, 1996b, 1996c); interstitial infiltrates at ≥0.44 or 0.44 mg Ni/m^3 as nickel subsulfide or nickel sulfate, respectively (NTP 1996b, 1996c), and fibrosis at 0.44 and 0.88 mg Ni/m^3 as nickel sulfate or nickel subsulfide, respectively (NTP 1996b, 1996c). As with rats, minimal alveolar macrophage hyperplasia was not considered adverse. The highest NOAEL values for respiratory effects in mice exposed to nickel sulfate, nickel subsulfide, and nickel oxide for intermediate durations were 0.22, 0.22, and 3.9 mg Ni/m^3, respectively (NTP 1996a, 1996b, 1996c).

Chronic-duration exposure to nickel (6 hours/day, 5 days/week for 2 years) resulted in chronic active lung inflammation (e.g., pneumonitis) in rats and mice at 0.06 mg Ni/m^3 as nickel sulfate, in rats at 0.11 mg Ni/m^3 and higher as nickel sulfide (NTP 1996b; Ottolenghi et al. 1975), in mice at 0.44 mg Ni/m^3 and higher as nickel subsulfide (NTP 1996b), in rats at 0.2 mg Ni/m^3 and higher as nickel oxide (NTP 1996a; Tanaka et al. 1988), and in mice at 1 mg Ni/m^3 as nickel oxide (NTP 1996a). Additional lung effects that

were found at the same dose levels as inflammation included alveolar epithelium hyperplasia (or bronchiolization), fibrosis in rats and mice exposed to nickel subsulfide (NTP 1996b), and bronchiolization and/or alveolar proteinosis in mice exposed to nickel oxide (NTP 1996a; Takenaka et al. 1985). Apart from the NTP (1996c) study of nickel sulfate in rats, NOAEL values for respiratory effects following chronic-duration exposure were not identified. The NOAEL of 0.03 mg Ni/m^3 and LOAEL of 0.06 mg Ni/m^3 identified in rats exposed to nickel sulfate (NTP 1996c) were used to derive a chronic-duration inhalation MRL for nickel.

The NTP (1996a, 1996b, 1996c) studies allow for the comparison of the toxicity of nickel sulfate, nickel subsulfide, and nickel oxide in rats and mice. Following acute- or intermediate-duration exposure, the toxicity of the different nickel compounds is related to its solubility, with soluble nickel sulfate being the most toxic and insoluble nickel oxide being the least toxic. The difference in the toxicity across compounds is probably due to the ability of water-soluble nickel compounds to cross the cell membrane and interact with cytoplasmic proteins. In contrast, the severity of inflammatory and proliferative lesions following chronic-duration exposure was greater in rats exposed to nickel subsulfide or nickel oxide, as compared to nickel sulfate. Additionally, parenchymal damage secondary to inflammation was evident in the rats exposed to nickel subsulfide and nickel oxide, but not nickel sulfate. For all durations and nickel compounds evaluated, rats appear to be more sensitive to the lung effects than mice; significant increases in the incidence of chronic lung inflammation were observed at lower concentrations in the rats than mice. Intermediate-duration studies (Benson et al. 1995a; Horie et al. 1985) that monitored animals for months after exposure termination suggest that nickel-induced lung damage is not readily reversible after exposure termination. In the Benson et al. (1995a) studies, alveolitis was observed in rats exposed to 0.11 mg Ni/m^3 as nickel sulfate and 1.96 mg Ni/m^3 as nickel oxide at the end of the 6-month exposure period and 4 months after exposure termination. Horie et al. (1985) reported localized interstitial pneumonia in rats exposed 6 hours/day, 5 days/week to 0.5 mg Ni/m^3 as nickel oxide for 1 month. Twelve and 20 months after termination of exposure to 6.3 mg Ni/m^3, squamous metaplasia of the bronchial epithelium, hyperplasia of the bronchial gland, and chronic bronchitis were observed.

In addition to the lung effects, several studies have demonstrated that exposure to nickel sulfate or nickel subsulfide can induce atrophy of the nasal olfactory epithelium (Evans et al. 1995; NTP 1996b, 1996c). The nasal lesions are typically observed at higher concentrations than the lung effects. In a study designed specifically to examine the effects of nickel on the olfactory system, rats were exposed to nickel sulfate at 0 or 0.635 mg Ni/m^3 6 hours/day for 16 days (Evans et al. 1995). Histological changes in the olfactory epithelium of exposed rats included a slight reduction in the number of bipolar sensory receptor cells, a decrease in the thickness of the olfactory epithelium resulting from a loss of sustentacular cells, a thinning

of apical cytoplasm, and a reduction in the number of sensory cilia on the surface of the cells. After a recovery period of 22 days, fewer sensory cilia were the only change that remained, indicating that the effects of an intermediate-duration exposure to nickel were reversible.

Oral

A case-series examined 20 female patients who presented with chronic rhinitis (nasal inflammation) and upon allergen testing all females only had a positive reaction to nickel sulfate in patch testing (Brera and Nicolini 2005). Authors suggest the rhinitis was due to nickel allergy further demonstrated by reduced nasal and bronchial symptoms in patients who had accepted a “strict and prolonged diet low in nickel content.”

Irregular respiration was one of several clinical signs of nickel toxicity observed in 4 out of 6 rats exposed to doses of nickel sulfate hexahydrate ≥111.6 mg Ni/kg/day for 3 days (Oller and Erexson 2007). Pneumonitis was observed in 6/19 male rats and 9/17 female rats treated for 91 days by gavage with 8.6 mg Ni/kg/day as nickel chloride (American Biogenics Corporation 1988). Significant increases in absolute and relative lung weights were observed in rats exposed to 28.8 mg Ni/kg/day as nickel sulfate in drinking water for 13 weeks (Obone et al. 1999). This study also found alterations in enzyme activity in bronchoalveolar lavage (BAL) fluid and lung tissues, including increases in protein levels in BAL fluid at 14.4 mg Ni/kg/day and higher, decreases in alkaline phosphatase activity in BAL fluid at 5.75 mg Ni/kg/day and higher, and decreases in alkaline phosphatase activity in lung tissue at 28.8 mg Ni/kg/day. No histological alterations were observed in the lungs. The study authors suggested that the decrease in alkaline phosphatase activity was indicative of decreased activity of type II alveolar cells and the increased total protein was indicative of increased air-blood barrier permeability. In a multigeneration study (RTI 1988a, 1988b), increased relative lung weights were observed in rats provided with nickel chloride in the drinking water at 55 mg Ni/kg/day, and an increase in cellular infiltration of the lungs was observed at 20 mg Ni/kg/day. Emphysema, bronchiectasis, and cholesterol granulomas were also observed in dogs exposed to 62.5 mg Ni/kg/day as nickel sulfate in the diet for 2 years, but not in rats exposed at up to 187.5 mg/kg/day for 2 years (Ambrose et al. 1976).

Dermal

Scratch tests and intradermal tests performed on a patient diagnosed with nickel-related asthma resulted in respiratory distress indicated by a more severe response to the tests when compared to the results from non-asthmatic controls (McConnell et al. 1973).

No studies were located regarding adverse respiratory effects in animals after dermal exposure to nickel.

2.5 CARDIOVASCULAR

Inhalation

No increases in the number of illness or deaths from cardiovascular diseases were reported in workers exposed to nickel (Cavallari et al. 2008; Cornell and Landis 1984; Cox et al. 1981; Cragle et al. 1984). A cross-sectional population level study in southern California reported a correlation between nickel concentrations in ambient air and mortality from ischemic heart disease (Cahill et al. 2011). Several population-level studies report an association of nickel concentration in air and cardiovascular hospitalizations, illness, and indicators (Bell et al. 2009; Bell et al. 2014; Huang et al. 2017; Jacobs et al. 2012; Niu et al. 2013; Occelli et al. 2020; Spiezia et al. 2016; Wu et al. 2012). In other epidemiological studies, no evidence of an association between nickel exposure and pulmonary embolism was seen (Spiezia et al. 2014), and between nickel exposure in ambient air and coronary events (Wolf et al. 2015). Epidemiological studies examining cardiovascular effects and exposure to nickel in ambient air are summarized in Table 2-4.

Microscopic examinations of the hearts of Fischer-344 rats exposed to nickel oxide, nickel subsulfide, or nickel sulfate for 12 6-hour exposures over 16 days did not reveal any changes at concentrations as high as 23.6, 7.33, or 12.2 mg Ni/m^3, respectively (NTP 1996a, 1996b, 1996c). Similarly, no changes were observed in B6C3F1 mice exposed to nickel oxide or nickel sulfate at concentrations as high as 23.6 or 1.4 mg Ni/m^3, respectively (NTP 1996a, 1996c). Acute-duration exposure in beagle dogs to nickel sulfate and nickel oxide at 0.1 and 0.06 mg Ni/m^3, respectively did not cause any effects in the cardiovascular system based on electrocardiogram test evaluations (Muggenburg et al. 2003).

No cardiovascular effects were observed in rats or mice exposed to 0.44, 1.83, or 7.9 mg Ni/m^3 as nickel sulfate, nickel subsulfide, or nickel oxide, respectively, 6 hours/day, 5 days/week for 13 weeks (NTP 1996a, 1996b, 1996c). Continuous exposure to metallic nickel at 0.0004 mg Ni/m^3 in male ApoE mice for 14 weeks (5 days/week, 6 hours/day) caused vascular endothelial dysfunction indicated by increased aortic relaxation (Ying et al. 2013). At similar lower concentrations of exposure to 0.00017 mg Ni/m^3 as nickel sulfate in ApoE mice, exposure induced microcirculatory dysfunction indicated by increases in adherent and rolling monocytes in the microcirculation after a 3-month continuous exposure (5 days/week, 6 hours/day) (Xu et al. 2012).

Chronic-duration exposure (6 hours/day, 5 days/week) of rats to nickel sulfate, nickel subsulfide, or nickel oxide at concentrations up to 0.11, 0.73, or 2 mg Ni/m^3, respectively, or exposure of mice to, 0.22, 0.88, or 3.9 mg Ni/m^3, respectively, did not result in microscopic changes in the heart (NTP 1996a, 1996b, 1996c). Continuous exposure (6 hours/day, 5 days/week) of Fischer-344 rats to 0.63 mg Ni/m^3 as

nickel sulfide for 78 weeks also did not affect the microscopic appearance of the heart (Ottolenghi et al. 1975).

Overall, cardiovascular effects of exposure to any form of nickel for any duration did not show an effect in rats and mice of different strains except $ApoE^{-/-}$ mice (Ying et al. 2013; Xu et al. 2012). This strain of mice is deficient in apolipoprotein E which is implicated in cardiovascular diseases, and is used to study cardiovascular diseases (Meir and Leitersdorf 2004).

Table 2-4. Epidemiological Studies Examining Cardiovascular Outcomes in Humans Exposed to Nickel in Ambient Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments:	Outcomes and Limitations
Bell et al. 2009 **Study Type/Population:** Time-series population study linking two national datasets by county and by season and analyzed the long-term average concentrations of $PM_{2.5}$ chemical components for 2000 to 2005 and the risk ratios (RRs) of cardiovascular and respiratory hospitalizations for persons 65 or older associated with a 10 $\mu g/m^3$ increase in $PM_{2.5}$ total mass on the same day for 106 US counties from 1999 to 2005.	**Exposure:** Analyzed long-term average concentrations of $PM_{2.5}$ chemical components for 2000-2005 and RRs of cardiovascular and respiratory hospitalizations for persons 65 years or older associated with a 10 ug/m^3 increase in PM2.5 total mass on the same day for 106 US counties for 1999 through 2005. 20 metals were analyzed in total. **Inclusion/Exclusion Criteria:** Counties were selected based on data availability for $PM_{2.5}$ total mass and chemical components and had populations of 200,000 or more. **Covariates Considered/Other Regression Adjustments:** Analysis adjusted for daily temperature and dew point temperature for the previous 3 days' temperatures. Percent increase in nickel, elemental carbon, and vanadium were adjusted by other chemical components in the regression analysis and reported both with and without co-pollutants.	**Outcomes:** Counties with higher $PM_{2.5}$ content of nickel were found to have higher risk of cardiovascular and respiratory hospitalizations associated with short-term exposure to $PM_{2.5}$. Reported percent increases in health effects estimates for $PM_{2.5}$ lag 0 and risk of cardiovascular hospitalizations (19% increase) and respiratory hospitalizations (223%) per interquartile range increase in the fraction of $PM_{2.5}$ total mass for each component, with and without co-pollutant adjustment (listed without co-pollutant adjustment here). **Limitations:** The population criterion results in more urban counties. The analysis also includes 19 other metals, in addition to nickel. The result found in the outcome is true for elemental carbon (EC) and vanadium as well.
Bell et al. 2014 **Study Type/Population:** Time series population study analyzing the relative risks of cardiovascular and respiratory	**Exposure:** Filter samples for four counties in Connecticut and Massachusetts were analyzed for $PM_{2.5}$ elements. Source apportionment was used to estimate daily $PM_{2.5}$ contributions from sources (traffic, road dust, oil combustion, and sea salt, and regional sources, e.g., coal	**Outcomes:** Found association between nickel in $PM_{2.5}$ exposure and cardiovascular and respiratory illness hospitalizations. Higher contribution of nickel strengthens associations between $PM_{2.5}$ mass and cardiovascular hospitalization rates. A higher risk of

Table 2-4. Epidemiological Studies Examining Cardiovascular Outcomes in Humans Exposed to Nickel in Ambient Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments:	Outcomes and Limitations
hospitalizations associated with short-term exposure to $PM_{2.5}$ constituents and sources for the Medicare population (> 330,000 persons ≥ 65 years old) in a time-series analysis between February 2000 and August 2004 in 4 counties in Connecticut (3) and Massachusetts (1). Effect was measured supplementing EPA's Chemical Speciation Network for the 4 counties with data from X-ray fluorescence elemental analysis of $PM_{2.5}$ filters collected at five EPA monitoring sites in the sample states.	combustion). Associations between daily $PM_{2.5}$ constituents and sources and risk of cardiovascular and respiratory hospitalizations for the Medicare population (< 330,000 persons > 65 years of age) were estimated with time-series analyses between August 2000 and February 2004. 12 metals were analyzed in total. Mean nickel exposure was found to be 0.003, median 0.0020. Mean nickel exposure was 0.003, median 0.0020, and $PM_{2.5}$ total mass was 0.02%. **Inclusion/Exclusion Criteria:** Exposure for $PM_{2.5}$, constituents, and sources by analyzing filters used by regulatory agencies to measure $PM_{2.5}$ total mass and used those data to source apportionment analysis. Estimated weather variables for each county. Identified at-risk population of Medicare beneficiaries (≥ 65 years old) who resided in the counties studied and were enrolled in the Medicare fee-for-service plan during August 2000 – February 2004. Included only emergency hospitalizations and used date of admission to calculate daily number of admissions and used the principal discharge diagnosis code as cause of admission. Days with missing data were omitted from the analysis.	respiratory hospitalizations was associated with higher levels of nickel, more than other pollutants examined. **Limitations:** Samples were taken every 3 days in some monitoring sites, and every day, missing some periods, in others. The authors cited several limitations, including the limited period of the data set prohibited the authors' extensive analysis by season; lack of key data for particle sources and constituents (e.g., ammonium sulfate); and minimum detection limits hindered the authors' ability to estimate exposure for all constituents and incorporate them in source-apportionment methods. Authors cited limitations also include confounding by covarying constituents and $PM_{2.5}$ in situations where $PM_{2.5}$ is associated with the health outcome. Nickel did not remain statistically significant when adjusted by black carbon.

Table 2-4. Epidemiological Studies Examining Cardiovascular Outcomes in Humans Exposed to Nickel in Ambient Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments:	Outcomes and Limitations
	Covariates Considered/Other Regression Adjustments: Analysis adjusted for co-pollutants.	
Cahill et al. 2011 **Study Type/Population:** Cross-sectional study analyzing the association of wintertime $PM_{2.5}$ mass with mortality associated with cardiovascular and specifically ischemic heart disease (IHD) in southern California Central Valley. Conducted an aerosol sampling transect in the study area during a 17-day period of strong stagnation. Mass and elemental components were measured.	**Exposure:** Authors conducted an aerosol sampling transect from Redding to Bakersfield during a 17-day period of strong stagnation, January 5-22, 2009. Mass and elemental components were measured every 3 hours in eight particle size modes, ranging from 10 to 0.09 µm, while ultrafine particles (<0.09 µm) were collected on Teflon filters. 32 elements were analyzed in this study. Over 6,400 measurements were made of mass and inorganic elements in nine size modes for the study period. **Inclusion/Exclusion Criteria:** Using meteorological predictions, the authors simultaneously sampled continuously by size, time, and composition for 17 days starting on January 5, 2009, at five sites from the extreme north to the extreme south of the study area. The study included three components, all conducted in winter conditions using the same equipment: 1) an initial year-long study of the DRUM sampler and the ARB's FRM to establish equivalency 2) a simultaneous transect across a heavily traveled secondary street to identify very fine and ultrafine aerosols from roadways, and 3) the main transect study in winter, 2009.	**Outcomes:** A correlation ($r^2 = 0.95$) was found between nickel and IHD mortality for concentration (ng/m^3) of very fine (0.09-0.26 µm) aerosols, and $r^2 = 0.70$ for concentration of ultrafine (<0.09µm) aerosols. **Limitations:** The authors state that the evidence they present in the study is not conclusive, but strongly supports the hypothesis that very fine and ultrafine transition metals (including nickel) are a causal factor in IHD in the Central Valley of California. The authors cited limited information on ultrafine metals from vehicular exhaust.

Table 2-4. Epidemiological Studies Examining Cardiovascular Outcomes in Humans Exposed to Nickel in Ambient Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments:	Outcomes and Limitations
	Covariates Considered/Other Regression Adjustments: Authors did not explicitly list any covariates or adjustments, though ancillary studies were performed including direct upwind-downwind profile across a heavily traveled secondary street near a stoplight (in which there would be braking, therefore exposure to brake drums and pads).	
Cavallari et al. 2008 **Study Type/Population:** Prospective panel study (cohort) examining the association between daytime exposure to the metal content of $PM_{2.5}$ and night heart rate variability (HRV) in a panel study of 26 male boilermaker construction workers exposed to metal-rich welding fumes. Authors recruited boilermakers between 1999 and 2006 at an apprentice welding school to participate in ECG monitoring over two 24-hour periods on both a workday and a non-workday.	**Exposure:** 26 male workers in boilermaker construction were monitored by ambulatory electrocardiogram (ECG) on a workday while exposure to welding fume and a non-workday (baseline) from 2004-2006. Exposure was analyzed by x-ray fluorescence for elemental content. Mean nickel exposure (n=31) was 0.11 $\mu g/m^3$ for personal, workday $PM_{2.5}$ measurement. 8 metals were analyzed. Each metal was modeled separately due to the small sample size. **Inclusion/Exclusion Criteria:** Included boilermaker construction workers exposed to metal-rich welding fumes. **Covariates Considered/Other Regression Adjustments:** Metal exposure was assessed both with and without adjustment for total $PM_{2.5.}$ Authors controlled for individual cardiac risk	**Outcomes:** The study did not observe a statistically significant association between nickel exposure and altered heart rate variability. Mean nickel exposure (n=31) was 0.11 $\mu g/m^3$ for personal, workday $PM_{2.5}$ measurement. The authors reported a regression coefficient (β) expressed as change in msec of night rMSSD (square root of the mean squared differences of successive intervals) per 1 $\mu g/m^3$ increase in exposure after adjusting for baseline HRV, smoking status, and with or without adjustment for total $PM_{2.5}$. Authors report β = -4.76 (not statistically significant) for nickel, adjusted for baseline night rMSSD and smoking status; β=1.03 (not statistically significant) with nickel and $PM_{2.5}$, adjusted for baseline night rMSSD and smoking status, and -0.006 (statistically significant with $p<0.05$) for particulates.

Table 2-4. Epidemiological Studies Examining Cardiovascular Outcomes in Humans Exposed to Nickel in Ambient Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments:	Outcomes and Limitations
From 2004 to 2006, 26 boilermakers were selected for monitoring for workday $PM_{2.5}$ exposure, which was then analyzed by x-ray fluorescence for elemental content. The 26 boilermakers were monitored a total of 31 times.	factors such as age and health status. All models were adjusted for cigarette smoking. Since metal and total $PM_{2.5}$ mass exposure covaried, authors investigated the effect of each metal, independent of $PM_{2.5}$ by including $PM_{2.5}$ in the model along with the metals.	**Limitations:** Of the 31 exposure measurements, 12 (39%) nickel samples had concentrations below the limit of detection. The authors cite exposure source as a major limitation of the study because it differs substantially from ambient $PM_{2.5}$ or other sources of $PM_{2.5}$. The metal component alone did not account for the observed declines in night HRV, suggesting the importance of other PM elemental components. Due to the small sample size, authors were unable to investigate the potential modifying effects of hypertension or cardiac compromises. A self-reported questionnaire was used to collect information on medical history, current cardiopulmonary symptoms, medication use, demographics, occupational history, and lifestyle factors such as smoking history.
Huang et al. 2017 **Study Type/Population:** A time-stratified case crossover study between fine particulate matter ($PM_{2.5}$) elemental composition and emergency admission to Third Xiangya Hospital of Central south	**Exposure:** Authors analyzed the correlation between emergency admissions for cerebral hemorrhage, cerebral infarction, TIA, coronary heart disease and $PM_{2.5}$, concentrations of chemical element compositions ($PM_{2.5}$), and PM_{10} in Changsha city from June 1, 2009, to October 31, 2009. The analysis of $PM_{2.5}$ elemental composition was performed by Energy Dispersive	**Outcomes:** Concentration rises of nickel for $PM_{2.5}$ in Changsha city were related to the increase of emergency admissions with hypertensive cerebral hemorrhage. The average mass concentration levels of $PM_{2.5}$ in Changsha city for nickel was reported as 40.72 ng/m^3. $PM_{2.5}$ element concentrations of nickel and emergency treatment OR values of hypertension associated with cardiovascular

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Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments:	Outcomes and Limitations
University with cardiovascular disease in Changsha city, China. The study analyzes data of emergency admissions from June 1, 2009, to October 31, 2009, and meteorological data from routine monitoring within the same time period. N = 1,027, with 86 cases of hypertension, 99 cases of cerebral hemorrhage, 353 cases of cerebral infarction, 242 cases of transient ischemic attack (TIA), and 246 cases of coronary heart disease.	X-Ray Fluorescence (EDXRF). 18 elements were measured in this analysis. **Inclusion/Exclusion Criteria:** Emergency admissions to Third Xiangya Hospital of Central South University with cardiovascular disease, including cerebral hemorrhage, cerebral infarction, TIA, and coronary heart disease from June 1, 2009, to October 31, 2009. **Covariates Considered/Other Regression Adjustments:** Authors adjusted for everyday air temperature, air pressure, and maximum wind speed for the selected $PM_{2.5.}$ Control cases were matched by day of the week to control any weekly patterns in emergency admissions and air pollution levels.	disease for each additional one IQR were reported. For hypertension, OR = 1.016; cerebral hemorrhage, OR = 1.826 (significant at p <0.5); cerebral infarction, OR = 1.169; TIA, OR = 1.277; coronary heart disease, OR = 1.184; total cardiovascular diseases, OR = 1.204. **Limitations:** Cases came from a single location. The study did not take socio-economic factors into account. The study did not adjust for body mass index, smoking, or comorbidities. $PM_{2.5}$ was only monitored for 5 months, a comparatively short time frame.
Jacobs et al. 2012 **Study Type/Population:** Cross-section panel study in persons living in five elderly homes in Antwerp, Belgium between June 2007, and October 2009. N = 88 non-smoking persons. Authors collected blood pressure and a blood sample two times on	**Exposure:** $PM_{2.5}$ samples were collected indoors, in a common room, and outdoors over approximately 24 hours. $PM_{2.5}$ samples were collected on glass or quartz filters immediately outside each elderly home. Authors performed pollutant-specific, exposure-response analysis. Data for $PM_{2.5}$ samples were collected over 39 days. The mean concentration of nickel in outdoor settings over 24 hours was 3.5 ng/m^3, and in indoor settings it was 2.5 ng/m^3.	**Outcomes:** in Model 2, nickel was significantly associated with elevated systolic blood pressure and pulse pressure among individuals on antihypertensive medication. The estimated mean change in systolic blood pressure values for an IQR increase in outdoor $PM_{2.5}$ elemental concentrations was reported in both model analyses. Among individuals with no antihypertensive medication, nickel concentration of outdoor $PM_{2.5}$ was related to

Table 2-4. Epidemiological Studies Examining Cardiovascular Outcomes in Humans Exposed to Nickel in Ambient Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments:	Outcomes and Limitations
two separate days. Authors also measured the elemental content of indoor and outdoor $PM_{2.5}$ and outdoor PM_{10}. Results were separated by persons taking antihypertensive medication (n=57) and in persons not using antihypertensive medication (n=31). Study staff measured systolic and diastolic blood pressure and heart rate on two separate visits. Pulse pressure (systolic blood pressure minus diastolic blood pressure) was also considered in the analyses. The analyses used the average of the last three of five consecutive blood pressure measurements.	**Inclusion/Exclusion Criteria:** Lived in one of five elderly homes under the same organization. Participants were 65 or older, non-smoking, and able to provide informed consent. **Covariates Considered/Other Regression Adjustments:** Two analyses were conducted: Model 1 and Model 2. The Model 1 analysis was adjusted for sex, age, body-mass index, period (the visit a measurement was taken), and outdoor temperature. The Model 2 analysis was adjusted for all factors included in Model 1, in addition to systolic and diastolic blood pressure.	non-significant decreases in systolic blood pressure. Estimated mean changes of 0.41 ng/m^3 (Model 1) and a 0.81 ng/m^3 (Model 2) were estimated for an IQR increase in outdoor nickel PM concentration. Among those on antihypertensive medication, the estimated mean systolic blood pressure change was 2.4 $\mu g/m^3$ (Model 1; non-significant) and 2.5 $\mu g/m^3$ (Model 2; significant). **Limitations:** 74 of the 88 participating people had a second clinical visit. The study did not have personal exposure measurements. The authors state they had a rather low number of participants and could not analyze the effects of PM on blood pressure for different medications. Authors also could not know for sure that participants took their antihypertensive medication the day of the examination.
Niu et al. 2013 **Study Type/Population:** Cross-sectional population study of non-smoking and healthy female 60–65-year-old residents in Jinchang and	**Exposure:** Daily $PM_{2.5}$ samples were collected from downtown areas of both Jinchang and Zhangye for a 12-month period. Personal sampling of $PM_{2.5}$ mass concentrations was conducted for the 60 subjects by use of a backpack containing a personal pump to collect $PM_{2.5}$ samples for 24 hours on days when blood	**Outcomes:** Nickel was significantly associated with ICAM-1 (a cardiovascular inflammatory biomarker), as was living in Jingchang which had a higher nickel concentration in air compared to Zhangye.

Table 2-4. Epidemiological Studies Examining Cardiovascular Outcomes in Humans Exposed to Nickel in Ambient Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments:	Outcomes and Limitations
Zhangye, China. Thirty women were recruited from each city. Authors conducted an examination of the difference in inflammatory biomarkers in subjects living in the two cities as a function of the levels of personal exposures to $PM_{2.5}$ and its chemical components, adjusting for individual risk factors. $PM_{2.5}$ measurements were collected over 12 months and from personal air monitoring. Blood samples were collected from each participant on the same day as personal air sampling however study authors did not specify the timing or frequency of collection.	samples were collected. Central ambient exposure monitoring in Jingchang resulted in Ni=204.8 ng/m^3, and 2.7 ng/m^3 in Zhangye. Personal exposure monitoring results were Ni=71.28 ng/m^3 and 4.88 ng/m^3, respectively. **Inclusion/Exclusion Criteria:** Elderly, non-smoking female residents ages 60-65 were first targeted. Men were excluded from this study because it was difficult to find non-smoking male subjects in these communities. Subjects with abnormal blood sugar and lipid profiles and who had diagnosed diseases, including CVD, diabetes and hypertension were excluded. **Covariates Considered/Other Regression Adjustments:** The authors adjusted models for individual risk factors, which included age, cotinine level, BMI, blood sugar, LDL, HDL, triglycerides, systolic and diastolic blood pressure. Metal concentrations were log-transformed.	**Limitations:** Relatively small sample size and males were excluded.
Occelli et al. 2020 **Study Type/Population:** Retrospective cohort population-level study. Authors assessed the	**Exposure:** Authors compared the spatial distributions of a composite air pollution index (SEnv) and the CHD rate after adjusting for the level of social deprivation. SEnv was calculated for neighborhoods from 20 spatialized environmental indicators, which included analysis	**Outcomes:** Overall, higher SEnv was positively associated with greater CHD risk (p=0.0151), and median nickel levels were positively associated with higher SEnv (SP = 0.22, p<0.0001). In the single-pollutant analysis, after adjustment of FDep, the relative

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Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments:	Outcomes and Limitations
relationship between exposure to multiple air pollutants and the incidence of coronary heart disease (CHD) in a general population sample collected in the Lille MONICA registry (2008-2011) on 3,268 incident cases (men and women 35-74 years old) from the French WHO. This data records all fatal and non-fatal CHD events, regardless of hospitalization. Authors derived a composite environmental score (SEnv) for cumulative exposure to air pollution, then used Poisson regression models to analyze associations between CHD rates and SEnv. Authors studied the Lille urban area in northern France; 473 neighborhoods were included in the analysis.	from lichen biomonitoring data to assess 16 metal loads and eutrophication, which together served as a guide to long-term overall air quality. Higher SEnv (tertile 3 the highest) indicate greater air pollution. Authors used the Fdep index, a deprivation index reflecting the spatial socioeconomic heterogeneity, validated in the French context that uses median household income, percentage of high school graduates aged 15 and over, percentage of blue-collar workers, and unemployment rate. The higher the Fdep index, the greater the level of deprivation. The median level of nickel for n=473 was reported as 2.86 µg/g. **Inclusion/Exclusion Criteria:** Authors only included incident coronary events. Of 5,448 cases from 2008-2011, n= 3,268. **Covariates Considered/Other Regression Adjustments:** Model was adjusted for age, sex area-level socio deprivation, and neighborhood spatial structure. Models included ecological covariates as fixed effects.	risk of CHD was 11% higher in neighborhoods in the highest tertile for nickel, compared to those in the lowest tertile (RR = 1.11, 95% CI: 1.00, 1.23). **Limitations:** Data on atmospheric pollutants (including heavy metals) came from different sources and were provided in various formats and units on various spatiotemporal scales. Authors could not take account of certain individual risk factors for CHD, such as smoking or diet. Study only uses data from women. Authors did not have data on incident cases' workplaces, which prevented authors from assessing their exposure to air pollution during the day.
Spiezia et al. 2014 **Study Type/Population:** Retrospective case-control	**Exposure:** Average mean concentrations of 10 pollutants were obtained from monitors located at 2 different sites in Padua. Nickel levels were evaluated using ambient concentration averages	**Outcomes:** There was no statistically significant difference in exposure between cases and controls. Authors report tertiles of exposure to air pollutants of the study

Table 2-4. Epidemiological Studies Examining Cardiovascular Outcomes in Humans Exposed to Nickel in Ambient Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments:	Outcomes and Limitations
study examining the associations between one month's exposure to elevated levels of different pollutants and the development of acute isolated pulmonary embolism (PE). The study group was 33 patients consecutively admitted to Padua Hospital with an objectively proven diagnosis of acute unprovoked isolated PE between January 2008 and October 2012. The control group consisted of 72 consecutive patients with objectively proven acute provoked isolated PE.	over the month preceding the index date (date of PE diagnosis). Nickel exposure was recorded in tertiles: $\leq$ 2.86 ng/m^3, 2.87-4.64 ng/m^3, and $\geq$ 4.65 ng/m^3 **Inclusion/Exclusion Criteria:** Only subjects with a "high probability" of PE at ventilation-perfusion scan were enrolled in the study. Patients were excluded if they were under anticoagulant treatment at the time of the diagnosis of PE, if they were under 18, if they exhibited a previous episode of PE, or if they were a resident outside the city of Padua. **Covariates Considered/Other Regression Adjustments:** The multivariate model was adjusted for age, gender, chronic obstructive pulmonary disease (COPD), smoking status, educational level, distance from monitoring stations, season, and temperature.	population during the month before enrollment. Tertiles were reported as $\leq$ 2.86 ng/m^3, accounting for 10 cases, 24 controls; 2.87-4.64 ng/m^3 accounted for 13 cases and 24 controls, and $\geq$ 4.65 ng/m^3 accounted for 10 cases (30%) and 24 controls (33%), all with p = 0.76. Study reported OR for isolated PE associated with an exposure to elevated air pollutants. At 4.65 ng/m^3, OR = 1.07 for univariate model, and OR = 0.60 for multivariate model. **Limitations:** Study has a relatively small sample size. Because of the number of variables included in the logistic regression analysis, the authors note the specific weight of each variable is questionable. The evaluation of the environmental air pollution was used as a surrogate measurement, which may result in an underestimation or overestimation of the personal exposure for each patient. The monitoring station does not fully consider the individual differences in the time spent at home and in other environments, such as workplaces or in traffic while commuting. Data on $PM_{2.5}$ levels were not available. Information on specific sources of pollution (e.g., factories, major roads) close to

Table 2-4. Epidemiological Studies Examining Cardiovascular Outcomes in Humans Exposed to Nickel in Ambient Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments:	Outcomes and Limitations
		the patient's home was not included in this study.

Table 2-4. Epidemiological Studies Examining Cardiovascular Outcomes in Humans Exposed to Nickel in Ambient Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments:	Outcomes and Limitations
Spiezia et al. 2016 **Study Type/Population:** Authors performed a retrospective case-control study to evaluate the association between short-term exposure to elevated levels of air pollution and the risk of developing an acute idiopathic proximal deep vein thrombosis (DVT) in the legs. All eligible patients were admitted between April 2010 and December 2012 to the thrombosis unit of the University of Padua (Italy) with acute symptoms indicative of DVT in the legs. 233 subjects with a diagnosis of acute proximal DVT in the legs were evaluated, and n = 220 patients were enrolled: 86 (39%) experienced unprovoked DVT, and 134 (61%) presented a provoked DVT (control group).	**Exposure:** All eligible patients were admitted between April 2010 and December 2012. Pollutants were measured over the month and trimester preceding the DVT diagnosis from two monitoring sites in Padua, which were obtained from the Regional Agency for Environmental Protection. Month = 4.00 ng/m^3 and trimester = 4.44 ng/m^3. Nickel was one of ten environmental pollutants studied (including metals). **Inclusion/Exclusion Criteria:** Patients under anticoagulant treatment at the time of the diagnosis of venous thromboembolism, or younger than 18, or with a previous episode of PE or DVT, or who were residents outside of the city of Padua were excluded. **Covariates Considered/Other Regression Adjustments:** Multivariate analysis was adjusted for age, gender, smoking status, educational level, distance from monitor stations, season, and temperature.	**Outcomes:** Authors reported estimated OR for unprovoked proximal DVT associated with elevated air pollutants (nickel) exposure. Month = 4.00 ng/m^3 and trimester = 4.44 ng/m^3. OR = 2.52 and 0.85 for univariate models for month and trimester, respectively. OR = 2.49 and 0.79 for multivariate for month and trimester estimates, respectively. Using the upper limit of the second tertile measured in controls in the month before DVT diagnosis as a cut-off point, authors found a 2.5-fold increase in the risk of unprovoked proximal DVT for individuals who were exposed to nickel levels equal/above the cutoff point in the month before DVT. **Limitations:** Study has relatively small sample size that can affect the precision of estimations. Evaluation of environmental air pollution was used as a surrogate measurement, causing a possible error in the estimation of personal exposure for each patient.

Table 2-4. Epidemiological Studies Examining Cardiovascular Outcomes in Humans Exposed to Nickel in Ambient Air

Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments:	Outcomes and Limitations
Wang et al. 2014 **Study Type/Population:** Retrospective cohort study. Analysis included 19 cohorts for 12 countries where PM measurements were available from north to south Europe (Finland, Norway, Sweden, Denmark, the Netherlands, Germany, the UK, Austria, Switzerland, France, Italy, and Greece). Population = 322,291 participants with 9,545 CVD deaths. CVD mortality was defined based on underlying cause of death recorded on death certificates. Three two-week measurements of PM2.5 and PM10 were conducted during different seasons between October 2008 and May 2011 at 20 sites in each cohort study area (1 year per study area). Land Use Regression (LUR) models were developed	**Exposure:** Authors a priori selected 8 elements (including other metals) reflecting major anthropogenic sources. Annual average elemental concentrations at the baseline residential addresses of study participants were estimated by LUR models. Model 1 (see **Covariates**) presents hazard ratios (HR) for an increase of 1 ng/m^3 for $PM_{2.5}$ Ni and 2 ng/m^3 for PM_{10} Ni. **Inclusion/Exclusion Criteria:** In a sensitivity analysis, authors excluded cohorts with a weight larger than 50% in the meta-analysis. **Covariates Considered/Other Regression Adjustments:** Model 1 was adjusted for age, gender, and calendar time. Model 2 added adjustments for smoking status, smoking intensity, smoking duration, environmental tobacco smoke, fruit intake, vegetable intake, alcohol consumption, body mass index, education level, occupational class, employment status, marital status. Model 3 as in model 2 also adjusting for area-level socioeconomic status.	**Outcomes:** Study reports no significant associations between CVD mortality and exposure to neither $PM_{2.5}$ nor PM_{10} Ni elemental constituents. Hazard ratios for all associations between PM Ni and CVD mortality included 1 in confidence intervals. **Limitations:** LUR models used for exposure assessment were based on air pollution measurements in the period 2009-2011 while cohort studies included in ESCAPE started in the past (1985-2007). Predictions for nickel $PM_{2.5}$ in LUR models were poor in several study areas due to lack of identification of a major nickel source in the analysis which may have underestimated effect estimates. General explanations for a lack of association between CVD mortality and PM may apply, including better medication and medical treatment and less incidence of smoking. Site selection was designed for estimating especially the health effects on traffic pollution, which may restrict the power to detect other emission sources.

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Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments:	Outcomes and Limitations
for each element to explain annual concentrations.		
Wolf et al. 2015 **Study Type/Population:** Retrospective cohort study of 11 European cohorts. 5,157 incident coronary events were identified within 100,166 persons followed for 1,154,386 person-years. Enrollment period was between 1992 and 2007. Mean age was between 44 and 74. Long-term residential concentrations of PM_{10} and $PM_{2.5}$ were estimated with land use regression models.	**Exposure:** A PM was measured based on standardized methodology between 2008 and 2011. In each study region, authors performed three 14-day measurement periods at 20 monitoring sites over approximately 1 year. Authors developed land use regression models for each area and each exposure variable. Authors used Cox proportional hazard models adjusted for a common set of confounders to estimate cohort-specific component effect. Other metals were analyzed in this analysis. **Inclusion/Exclusion Criteria:** The analyses were restricted to persons with no missing information in both the exposure variables and the covariates of the main model. Authors excluded persons with prevalent events. **Covariates Considered/Other Regression Adjustments:** The main model included year of enrollment, sex, marital status education, occupation, smoking status, smoking duration, smoking intensity among current smokers, and an area-level socioeconomic indicator.	**Outcomes:** Authors reported the association between incidence of coronary events and elemental composition in 11 European cohorts. However, incidence of coronary events did not appear associated with PM_{10} Ni or $PM_{2.5}$ Ni. The PM_{10} hazard ratios in the single and PM-adjusted constituent models were 1.13 (1.00,1.28) and 1.09 (0.94, 1.28), respectively. The $PM_{2.5}$ hazard ratios in the single and PM-adjusted constituent models were 1.10 (0.89, 1.37) and 1.07 (0.82, 1.39), respectively. **Limitations:** Specific predictor variables for sources such as biomass combustion were not available in the geographic databases that authors had access to. Fewer sites were included to capture differences in other sources, such as industry or ports. Many models did not contain specific source predictor variables, so authors could not disentangle effects of related elements. Because elements may stand for different sources in different regions, meta-analysis may not always be meaningful.

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Reference, Study Type, and Study Population	Exposure, Inclusion/Exclusion Criteria, and Covariates Considered/Other Regression Adjustments:	Outcomes and Limitations
Wu et al. 2012 **Study Type/Population:** In a prospective panel study of 17 nonsmoking male mail carriers recruited from the Sin-Jhuang Post Office, Taipei County, Taiwan. Subjects were followed for 5-6 days while delivering mail on motorcycles. The weekly campaigns were conducted over 7 weeks in February and March of 2007.	**Exposure:** Authors applied linear mixed-effects models with repeated health measurements to assess the relationship between cardiovascular effects and personal air pollution exposure. Each mail carrier wore a personal cascade impactor sampler with the air inlet in the breathing zone. The sampling pump was turned on only during periods where participants were delivering mail outdoors. Ambient PM samples were also collected at a central monitoring site near the post office. Mean exposure is between 0.8 and 2.4 ng/m^3 for subject and central monitoring sites. 20 metals were included in this analysis. **Inclusion/Exclusion Criteria:** Individuals with existing cardiovascular disease were excluded from participation in the study. **Covariates Considered/Other Regression Adjustments:** Authors controlled for fixed covariates of the subjects' age, body mass index, frequency of secondhand smoke exposure, and ambient temperature during the working period.	**Outcomes:** Nickel exposure was associated with a 2% decline in LF/HF ratio (an indicator of heart rate variability). There was no significant association with any of the other four heart rate indicators measured. **Limitations:** The exposure data of the 17 subjects were not representative of all mail carriers. The potential lag effects of PM exposures were not evaluated due to the limitation of having time-integrated filter samples. The study mainly focused on metal components of PM samples, when other hazardous compounds may be absorbed onto the surface of these particles and lead to certain health effects. Having only metal data limited the number of sources that could be separated by source apportionment models. It is possible some of the identified associations occurred by chance.

Oral

Nickel sulfate crystals (rough estimate of 570 mg Ni/kg) were accidentally ingested by a 2-year-old child (Daldrup et al. 1983). Four hours after ingestion, cardiac arrest occurred, and the child died 8 hours after exposure.

Rats exposed to 8.6 mg Ni/kg/day as nickel chloride for 91 days had decreased heart weight (American Biogenics Corporation 1988), whereas rats exposed to 75 mg Ni/kg/day as nickel sulfate for 2 years had increased heart weight (Ambrose et al. 1976). Because the changes in heart weight were not accompanied by histological changes and decreases in body weight gain were also observed, the significance of these changes is not known. Rats exposed by gavage for 21 days to 7.6 mg Ni/kg/day as nickel sulfate had an increase of atherogenic index, an index of triglycerides and high-density lipoprotein cholesterol, serving as indicators of cardiovascular disease (Adeyemi et al. 2017). Histological changes in the heart were not observed in rats treated with nickel chloride in the drinking water at 40 mg/kg/day for up to 30 weeks (RTI 1988a), rats exposed to 28.8 mg Ni/kg/day as nickel sulfate in drinking water (Obone et al. 1999), rats exposed to 187.5 mg Ni/kg/day as nickel sulfate in the diet for 2 years (Ambrose et al. 1976), rats administered via gavage 22 mg Ni/kg/day (males) or 33 mg Ni/kg/day (females) as nickel sulfate for 90 days (Springborn Laboratories 2002), or dogs provided with nickel sulfate in the diet at a dose of 62.5 mg Ni/kg/day for 2 years (Ambrose et al. 1976). No heart lesions were reported during gross necropsy of male and female rats exposed to 2.2 mg Ni/kg/day as nickel sulfate daily for 18 weeks (Springborn Laboratories 2000a).

Dermal

No studies were identified that examined adverse cardiovascular effects in humans or animals after dermal exposure to nickel.

2.6 GASTROINTESTINAL

Inhalation

No studies were identified that examined gastrointestinal effects in humans after inhalation exposure to nickel.

Histopathological examinations of the gastrointestinal tract of mice and rats exposed to nickel sulfate, nickel subsulfide, or nickel oxide for 6-hour exposures over 12 days did not reveal any changes at concentrations as high as 12.2, 7.33, or 23.6 mg Ni/m^3, respectively, in rats and 1.4, 3.65, or 23.6 mg Ni/m^3, respectively, in mice (NTP 1996a, 1996b, 1996c). Likewise, no histological alterations were

observed in the gastrointestinal tracts of rats and mice exposed to 0.44, 1.83, or 7.9 mg Ni/m^3 as nickel sulfate, nickel subsulfide, or nickel oxide, respectively, 6 hours/day, 5 days/week for 13 weeks (NTP 1996a, 1996b, 1996c). Chronic-duration exposure of rats to nickel sulfate, nickel subsulfide, or nickel oxide at concentrations up to 0.11, 0.73, or 2 mg Ni/m^3, respectively, or exposure of mice to 0.22, 0.88, or 3.9 mg Ni/m^3 as nickel sulfate, nickel subsulfide, or nickel oxide, respectively, did not result in microscopic changes in the gastrointestinal tract (NTP 1996a, 1996b, 1996c). Continuous chronic-duration exposure (6 hours/day, 5 days/week) of rats to 0.63 mg Ni/m^3 as nickel sulfide for 78 weeks also did not affect the microscopic appearance of the intestines (Ottolenghi et al. 1975).

Oral

Symptoms of gastrointestinal distress were most frequently reported by workers who drank water during one work shift from a water fountain contaminated with nickel sulfate, nickel chloride, and boric acid (Sunderman et al. 1988). The workers who reported symptoms were exposed to an estimated dose of 7.1–35.7 mg Ni/kg. Of the 32 workers exposed, 20 reported symptoms including nausea (15 workers), abdominal cramps (14 workers), diarrhea (4 workers), and vomiting (3 workers). The gastrointestinal symptoms persisted 1-2 days in 10 workers who were then hospitalized. Although the actual contribution of boric acid to these effects is not known, the investigators (Sunderman et al. 1988) indicate that the intake of 20–200 mg boric acid probably did not contribute to the observed effects because the effects of boric acid are generally observed only following ingestion of ≥4 g by adults.

Discolored gastrointestinal contents, ulcerative gastritis, and enteritis were observed in rats that died following treatment by gavage with 25 mg Ni/kg/day as nickel chloride hexahydrate for up to 91 days (American Biogenics Corporation 1988). Discolored (green) gastrointestinal contents were also observed at 1.2 and 8.6 mg/kg/day. The discoloration may have been due to the presence of nickel chloride in the gastrointestinal tract and is not considered an adverse effect. Adverse gastrointestinal effects were not observed in rats exposed to 28.8 mg Ni/kg/day as nickel sulfate in drinking water for 13 weeks (Obone et al. 1999), rats treated with nickel sulfate in the diet at 187.5 mg Ni/kg/day for 2 years (Ambrose et al. 1976), or rats receiving gavage doses of 22 (males) or 33 (females) mg Ni/kg/day as nickel sulfate (Springborn Laboratories 2002). During the first 3 days of a 2-year study, dogs vomited following treatment with nickel sulfate in the diet at 62.5 mg Ni/kg/day (Ambrose et al. 1976). The dose was lowered to 37.5 mg Ni/kg/day for 2 weeks, and then incrementally raised at 2-week intervals back to 62.5 mg/kg/day, at which time, no further gastrointestinal distress was noted. These studies indicate that high doses of nickel can be irritating to the gastrointestinal tract, although acclimation to high levels of dietary nickel can occur. The toxicological significance of the results of the American Biogenics Corporation

(1988) is not known, particularly since studies in rats (Ambrose et al. 1976; Obone et al. 1999; Springborn Laboratories 2000a, 2002) have not reported gastrointestinal effects.

Singla et al. (2006) exposed Wistar Albino male rats to 18.96 mg Ni/kg/day as nickel sulfate for 7 days daily and observed several changes in the intestines. Nickel-exposed animals had altered enzyme activity levels, specifically brush border enzymes along the crypt–villus axis, in the intestines compared to controls indicating an effect on digestive gut function.

Dermal

No studies were identified that examined adverse gastrointestinal effects in humans or animals after dermal exposure to nickel.

2.7 HEMATOLOGICAL

Inhalation

No studies were identified that examined hematological effects in humans after inhalation exposure to nickel.

Several hematological alterations were observed in studies by Weischer et al. (1980) and NTP (1996a, 1996b, 1996c). A decrease in hematocrit level was observed in male rats continuously exposed to 0.178 or 0.385 mg Ni/m^3 as nickel oxide for 28 days (Weischer et al. 1980); no significant alterations were observed at 0.785 mg Ni/m^3. The biological significance of a decrease in hematocrit level in the absence of hemoglobin or erythrocyte alterations is not known and lacks a clear dose-response. In non-pregnant females continuously exposed to nickel oxide for 21 days, increases in hematocrit and hemoglobin levels were observed at 0.8 mg Ni/m^3 and higher; an increase in mean cell volume and a decrease in erythrocyte levels were observed at 1.6 mg Ni/m^3 and higher (Weischer et al. 1980). Similarly, increases in hematocrit, hemoglobin, and erythrocyte levels were observed in rats exposed to nickel subsulfide at 0.73 mg Ni/m^3 6 hours/day, 5 days/week for 2 years (NTP 1996b). As noted by NTP (1996b), increases in hematocrit, hemoglobin, and erythrocytes are consistent with erythropoietin production in response to tissue hypoxia, possibly because of the nickel-induced lung damage. Chronic-duration exposure of rats to nickel oxide or nickel sulfate at concentrations up to 2 or 0.11 mg Ni/m^3, respectively, and chronic-duration exposure of mice to nickel oxide, nickel subsulfide, or nickel sulfate at concentrations up to 3.9, 0.88, or 0.22 mg Ni/m^3, respectively, did not result in significant hematological effects (NTP 1996a, 1996b, 1996c). Oller et al. (2008) observed increases in hemoglobin and hematocrit levels in rats after 78 weeks of exposure to concentrations ≥0.1 mg Ni/mg^3 of metallic nickel. These same rats showed labored breathing and chronic lung inflammation.

Oral

A transient increase in blood reticulocytes was observed in 10 workers who were hospitalized for gastrointestinal symptoms after drinking water during one work shift from a water fountain contaminated with nickel sulfate, nickel chloride, and boric acid (Sunderman et al. 1988). These workers were among 20 workers who reported symptoms following exposure and were hospitalized due to the 1–2-day persistence of clinical gastrointestinal symptoms. The workers who reported symptoms were exposed to an estimated dose of 7.1–35.7 mg Ni/kg. The contribution of boric acid to these effects is not known.

Rat studies have indicated that intermediate-duration exposure to ≥0.7 mg Ni/kg/day as various nickel salts produces hematological effects. Effects included a decrease in hemoglobin level in rats exposed to 25 mg Ni/kg/day as nickel acetate in the diet for 6 weeks (Whanger 1973), an increase in leukocyte levels in rats exposed to 0.49 mg Ni/kg/day as nickel chloride in drinking water for 28 days, but not at 0.97 mg Ni/kg/day (Weischer et al. 1980), and an increase in platelet counts in rats administered via gavage 8.6 mg Ni/kg/day as nickel chloride for 91 days (American Biogenics Corporation 1988). Rats exposed to 7.58 mg Ni/kg/day as nickel sulfate for 21 days showed altered blood chemistry including reduced plasma protein (Adeyemi et al. 2017). Two years of daily exposure to doses of nickel sulfate hexahydrate up to 11.16 mg Ni/kg/day in rats did not result in significant exposure-related changes in hematological parameters including hemoglobin and hematocrit levels (Heim et al. 2007). Twenty-eight days of exposure to 0.036 mg Ni/kg/day as nickel sulfate in mice resulted in changes in blood composition including reduced red blood cells and hemoglobin and increased white blood cell count (Dahdouh et al. 2016). No hematological effects were observed in rats treated with nickel sulfate in the diet at a dose of 187.5 mg Ni/kg/day for 2 years (Ambrose et al. 1976). Low hematocrit levels were observed in dogs after chronic-duration dietary exposure to 62.5 mg Ni/kg/day as nickel sulfate (Ambrose et al. 1976).

Dermal

No studies were identified that examined adverse hematological effects in humans after dermal exposure to nickel.

Hematocrit and hemoglobin levels were not affected in guinea pigs treated with 100 mg Ni/kg as nickel sulfate placed on skin of the back for 15 or 30 days (Mathur and Gupta 1994). Only one dose was examined in this study and there was no indication that the animals were prevented from licking the nickel from the skin; therefore, these effects could have resulted from oral exposure.

2.8 MUSCULOSKELETAL

Inhalation

No studies were identified that examined musculoskeletal effects in humans after inhalation exposure to nickel.

No histological alterations were observed in the bone of rats and mice exposed to nickel sulfate 6 hours/day for 12 days or 16 days (highest NOAEL is 12.2 mg Ni/m^3), 5 days/week for 13 weeks (0.44 mg Ni/m^3), or 5 days/week for 2 years (0.11 and 0.22 mg Ni/m^3 for rats and mice) (NTP 1996c); the muscles were not examined histologically in these studies. No alterations were observed in bone or muscle of rats and mice exposed to nickel oxide (6 hours/day, 5 days/week) at 23.6 mg Ni/m^3 for 16 days (12 days or 16 days), 7.9 mg Ni/m^3 for 13 weeks, or 2 (rats) or 3.9 mg Ni/m^3 (mice) for 2 years (NTP 1996a). Similarly, exposure to nickel subsulfide 6 hours/day, 5 days/week did not result in alterations in bone or muscle in rats at 7.33 mg Ni/m^3 for 13 weeks, 0.73 mg Ni/m^3 (rats) for 2 years, or mice at 7.33 mg Ni/m^3 for 16 days, 1.83 mg Ni/m^3 for 13 weeks, or 0.88 mg Ni/m^3 (mice) for 2 years (NTP 1996b).

Oral

Muscular pain was reported by one worker who drank water contaminated with nickel sulfate, nickel chloride, and boric acid during one work shift (Sunderman et al. 1988). This worker was among twenty workers who reported symptoms, primarily gastrointestinal, after 32 workers were exposed to an estimated dose of 7.1–35.7 mg Ni/kg. The contribution of boric acid to these effects is not known.

Microscopic changes in skeletal muscle were not observed in rats or dogs fed nickel sulfate in the diet at doses up to 187.5 mg Ni/kg/day for rats (Ambrose et al. 1976; Springborn Laboratories 2002) and 62.5 mg Ni/kg/day for dogs (Ambrose et al. 1976).

Dermal

No studies were identified that examined adverse musculoskeletal effects in humans or animals after dermal exposure to nickel.

2.9 HEPATIC

Inhalation

A prospective cohort study of nickel-plating workers found that nickel exposure affects hepatic inflammatory function (Kalahasthi et al. 2006). Workers (n=69) were grouped by no exposure, moderate, or high exposure indicated by nickel levels in blood, and the highest exposed group had significantly

elevated serum aspartate transaminase (AST) and serum alanine transaminase (ALT) levels (Kalahasthi et al. 2006). Only AST was elevated among workers in the moderate exposure group. This study is limited by lack of information on the exposure levels and the study authors did not provide information on possible exposure length.

No histological alterations were observed in the livers of rats or mice exposed to nickel subsulfide, nickel sulfate, or nickel oxide at concentrations of 7.33, 12.2, or 23.6 mg Ni/m^3, respectively, in rats and 1.4, 12.2, or 23.6 mg Ni/m^3, respectively, in mice exposed 6 hours/day, 12 days in a 16-day period (NTP 1996a, 1996b, 1996c), or 1.83, 0.44, or 7.9 mg Ni/m^3 6 hours/day, 5 days/week, for 13 weeks (NTP 1996a, 1996b, 1996c). Following chronic-duration exposure, no histological changes were observed in the livers of rats exposed to nickel sulfide at 0.63 mg Ni/m^3 (Ottolenghi et al. 1974) or 0.73 mg Ni/m^3 (NTP 1996b), to nickel oxide at 0.9 mg Ni/m^3 (Tanaka et al. 1988) or 2 mg Ni/m^3 (NTP 1996a), or to nickel sulfate at 0.11 mg Ni/m^3 (NTP 1996c). Chronic-duration exposure of mice to nickel oxide, nickel subsulfide, or nickel sulfate at concentrations up to 3.9, 0.88, or 0.22 mg Ni/m^3, respectively, did not result in microscopic changes in the liver (NTP 1996a, 1996b, 1996c).

Oral

A transient increase in serum bilirubin levels was observed in 3 of 10 workers who were hospitalized with primarily gastrointestinal symptoms after drinking water during one work shift from a water fountain contaminated with nickel sulfate, nickel chloride, and boric acid (Sunderman et al. 1988). The workers who reported symptoms or who were hospitalized (20 of 32) were exposed to an estimated dose of 7.1–35.7 mg Ni/kg. The contribution of boric acid to these effects is not known.

Decreased liver weight was observed in rats exposed to 0.97−75 mg Ni/kg/day as nickel chloride or nickel sulfate for 28 days to 2 years (Ambrose et al. 1976; American Biogenics Corporation 1988; Obone et al. 1999; Weischer et al. 1980) and mice exposed to 150 mg Ni/kg/day as nickel sulfate in drinking water for 180 days (Dieter et al. 1988). Adeyemi et al. (2017) observed changes in liver enzymes and histopathological changes following daily exposure for 21 days to 7.58 mg Ni/kg/day as nickel sulfate. Kamal et al. (2012) observed altered liver enzyme levels in rats exposed for 28 days to 3.81 mg Ni/kg/day as nickel sulfate hexahydrate in drinking water. Livers from nickel-exposed rats showed inflammation and cellular degeneration, and significant increases in activity of alanine transaminase, aspartate transaminase, alkaline phosphatase, and malondialdehyde, and decreased glutathione (Adeyemi et al. 2017). In mice exposed to nickel chloride daily for 40 days, histological examination of the liver showed diffuse cytoplasm and nuclei damage in hepatic cells following exposure to 0.905 mg Ni/kg/day (Gathwan et al. 2013). Among mice exposed to the higher dose of 7.2 mg Ni/kg/day, the livers showed

more serious damage including hepatocellular degeneration and hypertrophy of nuclei and blood in the central canal of the liver (Gathwan et al. 2013).

No alterations in absolute liver weights were observed in male and female rats administered via gavage 22 or 33 mg Ni/kg/day as nickel sulfate, respectively, for 90 days (Springborn Laboratories 2002); no histological alterations were reported in this study. Similarly no histological changes were reported in the livers of rats exposed for 18 weeks to doses of up to 2.2 mg Ni/kg/day (Springborn Laboratories 2000a). A significant increase in relative liver weight, however, was observed in dogs exposed to 62.5 mg Ni/kg/day as nickel sulfate for 2 years (Ambrose et al. 1976). Since histological changes in the liver were not observed in these studies and decreases in body weight gain were often observed at the same dose levels, the significance of changes in the liver-to-body weight ratios are unclear.

Dermal

No studies were identified that examined adverse hepatic effects in humans after dermal exposure to nickel.

Effects on the liver were observed in rats treated dermally (lateral abdominal area) with daily doses of 60 mg Ni/kg/day as nickel sulfate for 15 or 30 days (Mathur et al. 1977). The effects included swollen hepatocytes and feathery degeneration after 15 days and focal necrosis and vacuolization after 30 days.

Increased Mg2+ ATPase activity was observed in the livers of guinea pigs treated with 100 mg Ni/kg as nickel sulfate placed on skin of the back for 15 or 30 days (Mathur and Gupta 1994). Acid phosphatase and glucose-6-phosphatase activities were increased only after 30 days of treatment. In both of these studies, there was no indication that the animals were prevented from licking the nickel from the skin; therefore, these effects could have resulted from oral exposure.

2.10 RENAL

Inhalation

Marked tubular necrosis was observed in the kidneys of a man who died of ARDS 13 days after a 90-minute exposure to a very high concentration, simulated by study authors to be 382 mg/m^3 of metallic nickel of small particle size (<1.4 µm) (Rendall et al. 1994). Several days after the exposure, urinary concentrations of nickel were 700 µg/L, in comparison to levels of <0.1-13.3 µg/L in persons not occupationally exposed to nickel (Sunderman 1993).

In nickel refinery workers, a significant association was found between increased levels of nickel in urine and increased urinary β2-microglobulin levels (Sunderman and Horak 1981). A significant increase in

urinary β2-microglobulin levels was observed in a group of workers with urinary nickel levels exceeding 100 µg/L; urinary β2-microglobulin levels were not significantly altered in workers with urine nickel levels of less than 100 µg/L. Urinary levels of total proteins, β2-microglobulin, retinol binding protein, and N-acetyl-β-D-glycosaminidase (NAG) were increased in 12 women, and urinary lysozyme and NAG were increased in 14 men occupationally exposed to soluble nickel (sulfate, chloride) compounds at an average concentration of 0.75 mg Ni/m^3 (Vyskocil et al. 1994a). Although the average exposure concentration was the same for women and men, women may have been more highly exposed as indicated by urine concentrations of 10.3 µg Ni/g creatinine in women compared to 5 µg Ni/g creatinine in men. The biomarkers of effect that were changed reflected tubular dysfunction. No effects on markers of glomerular function, urinary albumin levels, or transferrin levels were noted. Sanford and Nieboer (1992) did not find significant alterations in urinary β2-microglobulin levels in nickel refinery workers with urine nickel levels of less than 60 µg/L. Multiple 24-hour urine collections were collected from each participant. Sanford and Nieboer (1992) noted that elevated urinary β2-microglobulin levels were found in spot urine samples of three workers; however, when the levels were averaged over three or more voids (multiple samples from a participant), the average levels were within the normal range. A study of 17 electroforming workers did not find evidence of proteinuria (Wall and Calnan 1980).

No change in kidney weight was reported in rats exposed to 0.635 mg Ni/m^3 for 16 days, 6 hours/day, when compared to controls (Evans et al. 1995). No histological alterations were observed in the kidneys of rats or mice exposed to nickel sulfate, nickel subsulfide, or nickel oxide 6 hours/day, 5 days/week, at concentrations of ≤12.2, 7.33, or 23.6 mg Ni/m^3, respectively, for 16 days (12 days in a 16-day period) (NTP 1996a, 1996b, 1996c), or ≤0.44, 1.83, or 7.9 mg Ni/m^3, respectively, for 13 weeks (NTP 1996a, 1996b, 1996c), or 0.9 mg Ni/m^3 as nickel oxide for 12 months (Tanaka et al. 1988). Chronic-duration exposure of rats to nickel oxide (NTP 1996a; Tanaka et al. 1988), nickel subsulfide (NTP 1996b), or nickel sulfate (NTP 1996c) at concentrations up to 2, 0.73, or 0.11 mg Ni/m^3, respectively, did not result in histological alterations in the kidneys. Additionally, no alterations were observed in mice exposed to nickel oxide, nickel subsulfide, or nickel sulfate at concentrations up to 3.9, 0.88, or 0.22 mg Ni/m^3, respectively (NTP 1996a, 1996b, 1996c).

Changes in serum urea are reported in 21 and 28 day studies in male rats exposed to concentrations of 0.8 and 0.178 mg Ni/m^3 as nickel oxide, respectively (Weischer et al. 1980). In a chronic-duration 104-week study, male and female rat histopathology showed granular brown pigment in the kidneys (Oller et al. 2008). Incidence in females was significantly higher at concentrations ≥0.1 mg Ni/m^3 metallic nickel, while in males incidence increased at concentrations ≥0.4 mg Ni/m^3. A separate 78-80 week study in rats

did not observe any histopathological changes in either males or females at 0.63 mg Ni/m^3 as nickel sulfide (Ottolenghi et al. 1975).

Oral

A transient increase in urine albumin levels was observed in 3 of 10 workers who were hospitalized with primarily gastrointestinal symptoms after drinking water during one work shift from a water fountain contaminated with nickel sulfate, nickel chloride, and boric acid (Sunderman et al. 1988). Among 32 exposed workers, 20 reported symptoms and 10 had to be hospitalized due to the persistence of gastrointestinal symptoms. The workers who reported symptoms were exposed to an estimated dose of 7.1–35.7 mg Ni/kg. The contribution of boric acid to these effects is not known.

Cellular changes were observed in kidney sections of rats exposed to 0.7585 mg Ni/m^3 as nickel sulfate for 21 days (Adeyemi and Elebiyo 2014). These changes included swollen renal tubules, necrosis, and nephritis, and further there was a 12% decline in kidney-to-body weight ratio and increases in plasma creatinine and urea (Adeyemi and Elebiyo 2014). A 28-day study in male Swiss albino rats exposed to 0.036 mg Ni/m^3 as nickel sulfate reported histological findings of tubule degeneration and tubular necrosis among other lesions (Dahdouh et al. 2016). Renal dysfunction was further indicated by increases in serum urea, uric acid, and creatinine.

Renal tubular damage at the corticomedullary junction described as minor was observed in mice exposed to ≥108 mg Ni/kg/day as nickel sulfate in the drinking water for 180 days (Dieter et al. 1988). The renal effects included the loss of renal tubular epithelial cells and the presence of hyaline casts in the tubule (suggesting protein loss). No changes in markers of renal tubular function (urinary lactate dehydrogenase and NAG levels and β2-microglobulin levels) were observed in rats exposed to nickel sulfate in the drinking water for 6 months at a concentration that supplied doses of 6.9 mg/kg/day for males and 7.6 mg/kg/day for females (Vyskocil et al. 1994b). Urinary albumin levels, a marker of glomerular barrier dysfunction, was significantly increased in nickel-exposed female rats. Albumin excretion also tended to be higher in male rats but did not reach statistical significance because of two control rats with very high values. The investigators noted that male rats develop a spontaneous nephrosis as they age and that this may have obscured the effect of nickel. Significant decreases in urine volume and urine glucose levels and increases in relative kidney weight at 14.4 or 28.8 mg Ni/kg/day and increases in blood urea nitrogen (BUN) at 28.8 mg Ni/kg/day were observed in rats exposed to nickel sulfate in drinking water for 13 weeks (Obone et al. 1999); no changes in γ-glutamyl transpeptidase activity, NAG activities, or histological alterations were observed.

In dogs, polyuria and increased kidney weight were observed after exposure to 62.5 mg Ni/kg/day as

nickel sulfate for 2 years; however, renal effects were not observed in similarly treated rats (Ambrose et al. 1976). Several studies in rats have reported significant changes in kidney weights following exposure to 0.97–55 mg Ni/kg/day as nickel salts for 28 days to 9 months (American Biogenics Corporation 1988; RTI 1988b; Weischer et al. 1980). However, there was no consistency in direction of the change; some studies reported increases in kidney weights while others reported decreases. The toxicological significance of these data is not known. Additionally, no histological alterations were observed in the kidneys of male and female rats exposed to 22 or 33 mg Ni/kg/day, respectively, as nickel sulfate administered via gavage for 90 days (Springborn Laboratories 2002).

Dermal

Proteinuria was not observed in electroforming industry workers exposed to nickel. No information was provided on exposure level or nickel compound (Wall and Calnan 1980).

No gross or microscopic lesions were observed in the kidneys of rats treated dermally with ≤100 mg Ni/kg/day as nickel sulfate for 15 or 30 days (Mathur et al. 1977). Increased Mg^{2+} ATPase activity was observed in the kidneys of guinea pigs treated with 100 mg Ni/kg as nickel sulfate placed on skin of the back for 30 days (Mathur and Gupta 1994). No adverse effect was noted at 15 days, and dermal nickel exposure had no effect on kidney acid phosphatase or glucose-6-phosphatase activities. In these studies, there was no indication that the animals were prevented from licking the nickel from the skin; therefore, the animals could have been orally exposed.

2.11 DERMAL

Inhalation

No studies were located regarding dermal effects in humans following inhalation exposure. However, contact dermatitis in persons exposed to nickel compounds is one of the most common effects of nickel exposure. In addition, immunological studies indicate that dermatitis is an allergic response to nickel, and significant effects on the immune system have been noted in workers exposed to nickel.

Wistar rats exposed to ≥0.1 mg/m^3 for 104 weeks (5 days/week, 6 hours/day) showed exposure-related clinical signs including dermal atonia (Oller et al. 2008). Microscopic changes in the skin were not observed in rats or mice exposed to nickel as nickel sulfate, nickel subsulfide, or nickel oxide at concentrations up to 12.2, 7.33, or 23.6 mg Ni/m^3, respectively, for 6 hours/day for 12 days in a 16-day period (NTP 1996a, 1996b, 1996c) or 0.44, 1.83, or 7.9 mg Ni/m^3 6 hours/day, 5 days/week for 13 weeks (NTP 1996a, 1996b, 1996c). Chronic-duration exposure of rats to nickel sulfate, nickel subsulfide, or nickel oxide at concentrations up to 0.11, 0.73, or 2 mg Ni/m^3, respectively, or exposure of mice at

concentrations up to 0.22, 0.88, or 3.9 mg Ni/m^3, respectively, did not result in microscopic changes in the skin (NTP 1996a, 1996b, 1996c).

Oral

Contact dermatitis, which results from dermal exposure to nickel, is the most prevalent effect of nickel in the general population. Several studies indicate that a single oral dose of nickel given as nickel sulfate can result in a flare up of dermatitis in nickel sensitive individuals (Burrows et al. 1981; Christensen and Möller 1975; Cronin et al. 1980; Gawkrodger et al. 1986; Hindsén et al. 2001; Jensen et al. 2003; Kaaber et al. 1978; Veien et al. 1987). Observed effects included erythema on the body, worsening of hand eczema, and a flare-up at the patch test site. Although some of the older studies reported low LOAEL values (e.g., 0.009 mg Ni/kg), these studies have several design limitations including small sample size, the observation of placebo effects, and non-double-blind study designs (possibly introducing investigator bias). Two studies have used many test subjects and a double-blind study design. One month after patch testing, an oral challenge dose of 1.0 mg nickel as nickel sulfate (0.014 mg/kg) resulted in dermatitis in two of nine nickel-sensitive subjects (not significantly different than placebo incidence of 0/9); exposure to 4.0 mg nickel (0.057 mg/kg) resulted in dermatitis in nine of nine subjects (Hindsén et al. 2001). Similarly, an oral challenge of 0, 0.3, 1.0, or 4.0 mg nickel as nickel sulfate (0, 0.0043, 0.014, or 0.057 mg/kg) administered 1 month after patch testing resulted in dermatitis in 1/10, 4/10, 4/10, and 7/10 nickel-sensitized individuals, respectively; no cutaneous reactions were observed in healthy controls receiving an oral challenge dose of 0 or 4.0 mg nickel (Jensen et al. 2003). Although some sensitive individuals may react to very low oral doses of nickel, the threshold for dermatitis in nickel-sensitized individuals appears to be around 0.01 mg Ni/kg; a dose of approximately 0.06 mg Ni/kg will result in a response in the most sensitized individuals.

Nielsen et al. (1990) fed 12 women with hand eczema and known allergy to nickel a diet (oatmeal, soybeans, cocoa) with 5 times the normal level of nickel (about 0.007 mg/kg/day) for 4 days. An aggravation of hand eczema was found in 6 of 12 women by day 4 after the start of the challenge, and although excess nickel was excreted 2 days after the last treatment, further exacerbation of hand eczema was observed in 10 of 12 women by day 11. Diet was no longer tracked after day 4 of the challenge period, therefore it is not known whether participant diet affected the reported outcomes.

Intermediate-duration studies suggest that longer-term oral exposure can be tolerated by some nickel sensitive individuals and may even serve to desensitize some individuals. Jordan and King (1979) found flaring of dermatitis in only 1/10 nickel-sensitive women given nickel sulfate at 0.007 mg/kg/day for 2 weeks. Patch test responses to nickel were reduced in nickel-sensitive women given one weekly dose of

0.05 or 0.07 (but not 0.007) mg Ni/kg as nickel sulfate for 6 weeks (Sjövall et al. 1987). Santucci et al. (1994) gave increasing daily doses of nickel (0.01–0.03 mg/kg/day) as nickel sulfate to eight nickel sensitive women for up to 178 days. A significant clinical improvement in hand eczema was observed in all subjects after 1 month of treatment, and continued treatment resulted in healing of all dermal lesions except for those on the hands. Measurement of urine and serum nickel suggested a decrease in the absorption of nickel and an increase in the excretion of nickel with longer exposure. The Santucci et al. (1994) study indicates that a daily dose of 0.01–0.03 mg Ni/kg can be tolerated by some nickel-sensitive people and may also serve to reduce their sensitivity. Among 44 sensitive subjects treated with a regimen of 1–2 ng nickel sulfates every other day, or daily for up to 2–3 years, 7 subjects stopped the treatment for unspecified reasons, 7 had reactivation of symptoms, and complete (29) or partial (1) disappearance of symptoms for 2–4 years was observed in 30 subjects.

Oral exposure before sensitizing exposure may also help prevent nickel sensitization in some individuals. A study of 2,159 subjects examining the relationship between ear piercing and orthodontic treatment found that nickel sensitivity was reduced significantly when orthodontic treatment preceded ear piercing (23.3 versus 38.1%) (van Hoogstraten et al. 1991). The investigators hypothesized that the oral nickel exposure that occurred during orthodontic treatment helped prevent the sensitization that occurred following ear piercing with earrings containing nickel. Orthodontic treatment after ear piercing did not affect the risk of nickel sensitization. Further evidence that oral exposure to nickel before a sensitizing exposure can prevent hypersensitivity is provided by the observation that nickel sensitivity in mice could be consistently produced only when metal frames to cover the cages and metal water nipples that released nickel were replaced with glass covers and nipples free of nickel (van Hoogstraten et al. 1991). Oral treatment of guinea pigs with nickel sulfate (30 mg/week for 6 weeks) has also been shown to prevent dermal sensitization (van Hoogstraten et al. 1991). Skin exposure of guinea pigs to nickel (non-sensitizing contacts) before oral exposure was also shown to interfere with oral tolerance induction.

Histological changes in the skin have not been observed in rats treated by gavage with nickel chloride at a dose of 8.6 mg Ni/kg/day for 91 days (American Biogenics Corporation 1988), or in rats and dogs exposed to nickel sulfate in the diet for 2 years at doses of 187.5 and 62.5 mg Ni/kg/day, respectively Ambrose et al. 1976). These studies suggest that the skin is not affected by orally administered nickel in animals that have not been previously sensitized to nickel.

Dermal

Allergic contact dermatitis is a commonly reported effect in humans exposed to nickel. Contact dermatitis was found in 15.5% of approximately 75,000 individuals undergoing patch tests with nickel sulfate (5%

in petrolatum) (Uter et al. 2003). A pooled analysis of 20,107 patched tested individuals reported a prevalence of 11.4% among the general population (Alinaghi et al. 2019), indicating the prevalence is between 11-16%. Smaller scale studies report slightly higher frequencies: 19.1% of 542 subjects (Akasya-Hillenbrand and Ozkaya-Bayazit 2002), 21.2% of 1,729 subjects (Wantke et al. 1996), and 20.13% of 3,040 subjects (Simonetti et al. 1998). In the general population (a random sample of 567 people aged 15–69 years responding to a mailed screening questionnaire on respiratory allergy symptoms), 11% of the subjects had a positive reaction to nickel patch tests (Nielsen et al. 2002). Contact dermatitis in response to nickel exposure is more frequently observed in females, particularly younger females, than in males or older individuals (Thyssen and Menne 2010; Uter et al. 2003; Wantke et al. 1996). This increased prevalence appears to be related to previous nickel exposure rather than increased susceptibility. Prolonged exposure to nickel in consumer products, especially jewelry, rather than occupational exposure, is often a sensitizing source. An association has been observed between ear piercing and nickel sensitivity (Akasya-Hillenbrand and Ozkaya-Bayazit 2002; Dotterud and Falk 1994; Larsson-Stymne and Widström 1985; Meijer et al. 1995; Uter et al. 2003). The prevalence of nickel allergy was 9% among girls (age 8, 11, and 15; n=960) with pierced ears compared to 1% among girls without pierced ears. Girls with more than one hole in each ear were also more likely to be sensitive to nickel than girls with only one hole in each ear (19 versus 11%) (Larsson-Stymne and Widström 1985). In a study in schoolchildren age 7–12, the frequency of nickel allergy was 30.8% among girls with pierced ears and 16.3% among girls who did not have pierced ears (Dotterud and Falk 1994). Similarly, 14% of females with pierced ears developed nickel allergy compared to 4% in females without pierced ears (Nielsen et al. 2002). Among a group of Swedish men (age 18–24) completing military service, 4.6% with pierced ears reacted to nickel, while 0.8% who did not have pierced ears had a positive reaction to nickel (Meijer et al. 1995). Keczkes et al. (1982) has shown that sensitivity to nickel remains for many years. Fourteen people who tested positively for nickel sensitivity using nickel sulfate also tested positive 10 years later. However, the time interval between exposures can influence the degree of reactivity (Hindsén et al. 1997). A stronger reaction was found in nickel sensitized women when there was a 1-month period between nickel sulfate exposures compared to a 4-month period. This study also found a stronger reaction when nickel sulfate was applied to an area with previous allergic contact dermatitis.

Patch test studies in sensitive individuals using nickel sulfate have shown a dose-response relationship between the amount of nickel and the severity of the test response (Emmett et al. 1988; Eun and Marks 1990). In a study of 12 individuals, a nickel concentration of 0.0316% (316 ppm) in petrolatum resulted in dermatitis, while a concentration of 0.01% (100 ppm) did not produce adverse effects (Eun and Marks 1990). In aqueous solution, the nickel concentration of 0.0316% (316 ppm) did not result in dermatitis. Although most patch testing is done with nickel sulfate because it is less irritating than nickel chloride,

nickel alloys on the skin interact with human sweat, resulting in the release of nickel chloride. Therefore, nickel chloride is the more relevant form of nickel for examining threshold concentrations (Menné 1994). Menné and Calvin (1993) examined skin reactions to various concentrations of nickel chloride in 51 sensitive and 16 non-sensitive individuals. Although inflammatory reactions in the sweat ducts and hair follicles were observed at 0.01% and lower, positive reactions to nickel were not observed. To be scored as a positive reaction, the test area had to have both redness and infiltration, while the appearance of vesicles and/or a bullous reaction were scored as a more severe reaction. At 0.1%, 4/51 and 1/51 tested positive with and without 4% sodium lauryl sulfate. Menné et al. (1987) examined the reactivity to different nickel alloys in 173 nickel-sensitive individuals. With one exception (Inconel 600), alloys that released nickel into synthetic sweat at a rate of 1 μg/cm^2 /week produced strong reactions.

Nickel sensitivity has been induced in guinea pigs following skin painting or intradermal injection with nickel sulfate (Turk and Parker 1977; Wahlberg 1976; Zissu et al. 1987). As discussed in Section 3.2.2.2, nickel sensitivity can also be induced in mice if oral exposure to nickel is reduced (Möller 1984; van Hoogstraten et al. 1991).

Adverse effects on the skin were observed in rats treated dermally with ≥40 mg Ni/kg/day as nickel sulfate for 15 or 30 days (Mathur et al. 1977). The effects included distortion of the epidermis and dermis after 15 days and hyper keratinization, vacuolization, hydropic degeneration of the basal layer, and atrophy of the epidermis at 30 days. Biochemical changes in the skin (enzymatic changes, increased lipid peroxidation, and an increase in the content of sulfhydryl groups and amino nitrogen) were observed in guinea pigs dermally exposed to nickel sulfate for up to 14 days (Mathur et al. 1988; Mathur et al. 1992). Additive effects were observed when nickel sulfate was given in combination with sodium lauryl sulfate.

2.12 OCULAR

Inhalation

No studies were identified that examined ocular effects in animals after inhalation exposure to nickel.

Oral

In a pharmacokinetic study in humans, transient left homonymous hemianopsia (loss of sight in the same corresponding two left halves of the visual fields of both eyes) occurred in one male subject following ingestion of 0.05 mg Ni/kg as nickel sulfate in the drinking water (Sunderman et al. 1989b). No adverse effects were found in other subjects (n=9) when lower doses of 0.018 and 0.012 mg Ni/kg were used.

No treatment-related ophthalmological changes were observed in rats treated by gavage with 8.6 mg Ni/kg/day as nickel chloride for 91 days (American Biogenics Corporation 1988).

Dermal

No studies were identified that examined adverse ocular effects in humans or animals after dermal exposure to nickel.

2.13 ENDOCRINE

Inhalation

No studies were located regarding endocrine effects in humans following inhalation exposure to nickel.

Histological examinations did not reveal any changes in the adrenal glands, pancreas, parathyroid, pituitary, or thyroid glands in rats or mice exposed to nickel as nickel sulfate, nickel oxide, or nickel subsulfide for 12 days (6-hour exposure) over 16 days or for 6 hours/day, 5 days/week for 13 weeks (NTP 1996a, 1996b, 1996c). The NOAEL values for endocrine effects were 12.2, 23.6, and 7.33 mg Ni/m^3 in rats and mice exposed to nickel sulfate, nickel oxide, and nickel subsulfide, respectively, for the shorter duration study and 0.44, 7.9, and 1.83 mg Ni/m^3, respectively, for the 13-week study. In Fischer-344 rats exposed intermittently to nickel sulfide at 0.63 mg Ni/m^3 for 78 weeks, no histological changes were observed in the thyroid or adrenal glands (Ottolenghi et al. 1975). Increased incidences of benign pheochromocytoma were observed in female Fischer-344 rats exposed to 2 mg Ni/m^3 as nickel oxide for 2 years (5 days/week, 6 hours/day) (NTP 1996a).

No effects were observed in Fischer-344 rats exposed chronically to nickel sulfate at concentrations up to 0.11 mg Ni/m^3, or in mice exposed to nickel oxide, nickel subsulfide, or nickel sulfate at concentrations of 3.9, 0.88, or 0.22 mg Ni/m^3, respectively (NTP 1996a, 1996b, 1996c). Chronic-duration exposure to metallic nickel at 0.4 mg Ni/m^3 in male rats resulted in relative adrenal gland weight 89% higher than controls and correlated with increased incidence of pheochromocytomas (Oller et al. 2008). However, the authors noted that the pheochromocytomas were secondary to lung toxicity of nickel exposure. In female rats exposed to 0.4 mg Ni/m^3, the incidence of angiectasis in the adrenal glands was greater than controls (Oller et al. 2008).

Oral

No studies were identified that examined endocrine effects in humans after oral exposure to nickel.

Although histological changes were not observed, increases in pituitary weights were observed in male rats, but not female rats, treated with nickel chloride at doses ≥20 mg Ni/kg/day for up to 30 weeks (RTI 1986, 1988a, 1988b). The multigeneration study (RTI 1988a, 1988b) is confounded by a decrease in both food and water intake. Decreased prolactin levels were observed in female rats treated with 31 mg Ni/kg/day as nickel chloride in the drinking water throughout the breeding and lactation of two litters (11 weeks before breeding, 2-week rest period after weaning of the first litter, followed by a second breeding), but not at a 6.8-mg/kg/day dose (Smith et al. 1993). Histological examinations did not reveal any adverse effects in the pituitary, thyroid, and adrenal glands or in the pancreas of rats and dogs treated with nickel sulfate in the diet for 2 years at 187.5 mg Ni/kg/day for rats and 62.5 mg Ni/kg/day for dogs (Ambrose et al. 1976).

Dermal

No studies were identified that examined adverse endocrine effects in humans after dermal exposure to nickel.

2.14 IMMUNOLOGICAL

Inhalation

Several immunological effects have been reported in humans exposed to nickel. In 38 production workers exposed to nickel (compound not specified), significant increases in levels of immunoglobulin G (IgG), IgA, and IgM and a significant decrease in IgE levels were observed (Bencko et al. 1983; Bencko et al. 1986). Significant increases in other serum proteins, which may be involved in cell-mediated immunity (including α1-antitrypsin, α2-macroglobulin, ceruloplasmin), were also observed. The increase in immunoglobulins and serum proteins suggests that the immune system was stimulated by nickel exposure. Similar but less-pronounced effects were observed in eight workers with hard metal asthma attributed to cobalt exposure and who then underwent a bronchial provocation challenge to nickel sulfate (Shirakawa et al. 1990). A relationship between nickel and cobalt sensitization is further supported by the finding that nickel-reactive IgE antibodies were observed in all of the workers (Shirakawa et al. 1990).

Buxton et al. (2021) reported no nickel-exposure related immune effects in female ICR mice exposed whole-body 24-hours to concentrations ≤0.0801 mg Ni/m^3 as nickel chloride hexahydrate. Immune response was tested using sheep red blood cells (SRBC) in a splenic antibody forming cell (AFC) assay. Reductions in the number of spleen cells appeared concentration-dependent, however were not associated with decreases in spleen or thymus weight. Additionally, increases in Total Spleen Activity and Specific Activity were significant, however were normal and within biological variability for the mouse breed.

Taken together, the assay did reveal immunosuppressive effects due to nickel chloride hexahydrate exposure (Buxton et al. 2021).

At higher concentrations, alterations in innate (or non-specific) and acquired immunity have been observed in animals. Several studies examined alveolar macrophage functions. A significant reduction in macrophage phagocytic activity was observed in mice exposed to 0.5 to 0.66 mg Ni/m^3 as nickel chloride for 2 hours (Adkins et al. 1979a, 1979c) or exposed to 0.47 mg Ni/m^3 as nickel oxide or 0.45 mg Ni/m^3 as nickel subsulfide 6 hours/day, 5 days/week for 65 days (Haley et al. 1990). Haley et al. (1990) performed a pulmonary alveolar macrophage (PAM) phagocytosis immune function test to measure nickel immunotoxicity. Other alveolar macrophage alterations include decreased lysozyme activity in rabbits exposed to 0.6 mg Ni/m^3 as nickel chloride 6 hours/day, 5 days/week for 4– 6 weeks (Bingham et al. 1972; Johansson et al. 1989; Johansson et al. 1987; Johansson et al. 1988), alterations in macrophage production of tumor necrosis factor (Goutet et al. 2000; Morimoto et al. 1995), and morphological alterations. Morimoto et al. (1995) found increased production of tumor necrosis factor in rats exposed to 9.2 mg Ni/m^3 as nickel oxide 8 hours/day, 5 days/week for 4 weeks. In contrast, Goutet et al. (2000) found a decrease in tumor necrosis factor production in rats following a single intratracheal instillation of nickel sulfate. The conflicting results may be due to exposure route, duration, or concentration differences between the studies. Alveolar macrophages from rabbits exposed to 1 mg Ni/m^3 as metallic nickel 6 hours/day, 5 days/week for 3–6 months (Johansson et al. 1980) or 0.6 mg Ni/m^3 as nickel chloride 6 hours/days, 5 days/week for 4–6 weeks (Johansson et al. 1987) or 4 months (Johansson et al. 1989; Johansson et al. 1988) had increases in membrane-bound lamellar bodies. Exposure to metallic nickel also resulted in macrophages with smooth surfaces; the frequency of occurrence was duration-related (Johansson et al. 1980). Exposure to 0.1 mg Ni/m^3 metallic nickel for 104 weeks resulted in increased incidence of minimal-to-severe histiocyte infiltrate in bronchial lymph nodes and extramedullary hematopoiesis in the spleen (Oller et al. 2008). Xu et al. (2012) tested the lowest concentration in mice exposed to 0.00017 mg Ni/m^3 as nickel sulfate for 3 months, and immunohistochemical staining showed increased macrophages in epididymal white adipose tissue (eWAT) and in lung tissue sections.

Several studies have examined the relationship between nickel exposures and acquired immune function. A concentration-related increase in susceptibility to Streptococci infection was seen in mice exposed to nickel chloride ($\leq$ 0.5 mg Ni/m^3) for 2 hours and then infected either immediately or after a 24-hour recovery period (Adkins et al. 1979c). Increased susceptibility was indicated by an exposure-related increase in mortality and decrease in relative mean survival time in exposure groups when compared to simultaneously infected non-nickel exposed controls (Adkins et al. 1979c). Increased mortality and reduced survival time was also observed following a 2-hour exposure to 0.46 mg Ni/m^3 as nickel sulfate

(Adkins et al. 1979b). An additional group of mice, exposed to 0.66 mg Ni/m^3 as nickel chloride, developed septicemia from the Streptococci infection and had a reduced ability to clear the inhaled bacteria 96 hours after infection (Adkins et al. 1979a). Other studies have found an impaired response to sheep red blood cells (decrease in the number of antibody production spleen cells) in mice exposed to 0.25 mg Ni/m^3 as nickel chloride for 2 hours (Graham et al. 1978) or rats continuously exposed to 0.2 mg Ni/m^3 as nickel oxide for 4 weeks or 0.15 mg Ni/m^3 for 4 months (Spiegelberg et al. 1984). A decreased resistance to a tumor challenge was also observed in mice exposed to 0.45 mg Ni/m^3 as nickel sulfate 6 hours/day, 5 days/week for 65 days (Haley et al. 1990).

A significant portion of nickel that is removed from the lung enters the lymphatic system, often inducing damage to the lymph nodes. Lymphoid hyperplasia in the bronchial and mediastinal lymph nodes was observed in rats exposed to 1.4 mg Ni/m^3 as nickel sulfate (NTP 1996c) or mice exposed to 0.88 mg Ni/m^3 as nickel subsulfide (NTP 1996b) 6 hours/day for 12 days in a 16-day period; no effects were observed in rats exposed to 7.33 mg Ni/m^3 as nickel subsulfide (NTP 1996b), rats and mice exposed to 23.5 mg Ni/m^3 as nickel oxide (NTP 1996a), and mice exposed to 3.1 mg Ni/m^3 as nickel sulfate (NTP 1996c). In intermediate-duration studies, a 6 hour/day, 5 day/week exposure resulted in lymphoid hyperplasia in bronchial lymph nodes of rats exposed to 0.22, 0.22, or 2 mg Ni/m^3 as nickel sulfate, nickel subsulfide, or nickel oxide, respectively, and in mice exposed to 0.44, 0.88, or 2 mg Ni/m^3 as nickel sulfate, nickel subsulfide, or nickel oxide, respectively (NTP 1996a, 1996b, 1996c). Similarly, lymphoid hyperplasia was observed in the bronchial lymph nodes of rats exposed to 0.11, 0.11, or 0.5 mg Ni/m^3 as nickel sulfate, nickel subsulfide, or nickel oxide, respectively, and in mice exposed to 0.22, 0.44, or 1 mg Ni/m^3 as nickel sulfate, nickel subsulfide, or nickel oxide, respectively (NTP 1996a, 1996b, 1996c).

Oral

Dermatitis resulting from nickel allergy is well reported in the literature (see Section 2.11 for further discussion of allergic dermatitis following oral exposure).

Effects on the immunological system following exposure to 44 mg Ni/kg/day and higher as nickel sulfate in the drinking water for 180 days were assessed in mice (Dieter et al. 1988). Mild thymic atrophy was observed at 44 mg Ni/kg/day and higher and mild splenic atrophy was observed at 108 mg Ni/kg/day and higher. Although several tests of immune function were performed, only two alterations were found—decreased spleen cellularity at 150 mg Ni/kg/day and impaired lymphoproliferative response to the B-cell mitogen, Escherichia coli lipopolysaccharide (LPS), at 44 mg Ni/kg/day and higher; a marginal response to sheep red blood cells was also observed at 150 mg Ni/kg/day. No response to concanavalin A (con A), natural killer cell activity, or resistance to Listeria monocytogenes challenge were observed. In addition to

the immune function responses, exposure to nickel sulfate resulted in alterations in bone marrow, decreases in bone marrow cellularity at 108 mg Ni/k g/day and higher, decreases in granulocyte macrophage progenitor cells (CFU-GM) at 44 mg Ni/kg/day and higher, and multipotential stem cells (CFU-S) at 108 mg Ni/kg/day and higher. The stem cell alterations were associated with alterations in glucose-6-phosphate dehydrogenase activity—increased at 44 mg Ni/kg/day and decreased at 108 and 150 mg Ni/kg/day. Obone et al. (1999) reported alterations in T-cell and B-cell subpopulations in the thymus and splenic lymphocytes in rats exposed to nickel sulfate in drinking water for 13 weeks. In the spleen, the changes consisted of an increase in the total number of cells at 14.4 mg Ni/kg/day and a decrease at 28.8 mg Ni/kg/day; an increase in CD^{4+} T cells at 14.4 mg Ni/kg/day and a decrease at 28.8 mg Ni/kg/day; increases in CD^{8+} T cells at 14.4 and 28.8 mg Ni/kg/day; an increase in the number of B cells at 14.4 mg Ni/kg/day; and a decrease in the ratio of B cells to total cells at 14.4 mg Ni/kg/day. In the thymus, the changes consisted of an increase in the total number of cells at 14.4 mg Ni/kg/day and a decrease at 28.8 mg Ni/kg/day; an increase in CD^{4+} T cells at 14.4 mg Ni/kg/day and a decrease at 28.8 mg Ni/kg/day; a decrease in the ratio of CD^{4+} T cells to total cells at 28.8 mg Ni/kg/day; increases in CD^{8+} T cells at 5.75 and 14.4 mg Ni/kg/day and a decrease at 28.8 mg Ni/kg/day; increases in the ratio of CD^{8+} T cells to total cells at 5.75 mg Ni/kg/day and higher; and an increase in the number of B cells at 14.4 mg Ni/kg/day and a decrease at 28.8 mg Ni/kg/day. When challenged with Coxsackie virus B3, an enhanced inflammatory response was observed in the hearts of mice treated with nickel chloride in drinking water at 20.3 mg Ni/kg/day for 10–11 weeks (Ilbäck et al. 1994). Nickel treatment had no adverse effect on virus-induced lethality, spleen or thymus weights, or the number of cells in the spleen or thymus. Springborn Laboratories (2000a) observed no gross necropsy changes in the spleen or thymus of rats following 16-to-18-week daily exposures to doses of 0.22 to 2.2 mg Ni/kg/day as nickel sulfate hexahydrate. Gross and microscopic examinations of the spleen did not reveal any adverse effects in dogs fed 62.5 mg Ni/kg/day as nickel sulfate in the diet for 2 years (Ambrose et al. 1976).

Dermal

Contact dermatitis resulting from nickel allergy is well reported in the literature (see Section 2.11for further discussion of allergic reactions to nickel following dermal exposure). A relationship between human lymphocyte antigens (HLA) and nickel sensitivity was observed in individuals who had contact allergic reactions and positive results in the patch test (Mozzanica et al. 1990). The individuals had not been occupationally exposed to nickel. The HLA typing found a significantly greater prevalence of HLADRw6 antigen in the nickel-sensitive group compared to normal controls. The relative risk for individuals with DRw6 to develop a sensitivity to nickel was approximately 3.3. In individuals with allergic contact dermatitis to nickel, nickel directly bound and activated T-cells (Kapsenberg et al. 1988).

The dose-response relationship for the development of nickel sensitivity has been examined in a mouse model (Siller and Seymour 1994). The sensitization exposure involved placing a 6-mm pad containing 45 µL of a 0, 1, 5, 10, 15, or 20% nickel sulfate solution on the shaved abdominal skin of mice. This pad was left on the skin under occlusion for 7 days. Seven days after the sensitization procedure, the mice were challenged with 10 µL of a 0.4% aqueous nickel sulfate solution injected into the footpad. Saline was injected into the opposite footpad as a control. Contact hypersensitivity, indicated by footpad swelling, was elicited at all doses, although the degree of swelling was minimal at the 1% concentration. Footpad swelling increased as the sensitizing dose increased and generally peaked between 24 and 48 hours after the challenge. In a comparison of the responses between male and female mice, males showed a weaker and more variable response than females, and the response peaked at 72 hours in males compared to 48 hours in females.

Nickel-activated nuclear factor-kappa B (NF-κB) transcription factor may explain immune response to nickel contact resulting in nickel sensitivity (Kasprzak et al. 2003). NF-κB is involved in the inducible expression of adhesion molecules which are involved in leukocyte recruitment to inflammation sites (Goebeler et al. 1993; Kasprzak et al. 2003). In a skin dendritic cell line, nickel-induced activation of NF-κB transcription factor stimulated inducible isoform of nitric oxide synthase (iNOS) expression (Cruz et al. 2004); iNOS is involved in the regulation of immune responses. NF-κB activation by nickel also induces interleukin-8 (IL-8) production (Freitas et al. 2010) which plays a role in recruiting immune cells to inflammation sites.

2.15 NEUROLOGICAL

Inhalation

A single case of generalized tonic-clonic seizure was reported in a 43-year-old with no prior history to indicate a cause, and upon further examination that patient had elevated levels of nickel in urine (Denays et al. 2005). Acute nickel poisoning was then suspected as a coworker from the same workshop had been admitted a week prior with a first-time seizure and respiratory complaints. A retrospective case-control study of autistic children in California reported a potential association between autism and concentration of heavy metals in air, including nickel (Windham et al. 2006). The exposure to each metal was individually categorized into quartiles based on participant location. The fourth quartile (highest nickel exposure) of participants had significantly elevated adjusted odds of autism (OR=1.46; 1.04 – 2.06). Since the concentrations of many metals and solvents were correlated, the reported effect cannot be attributed to any specific exposure, and the modeled estimates only apply to the general geographic area and are not accurate to individual exposure (Windham et al. 2006).

Microscopic examinations did not reveal any changes in the whole brains of rats or mice exposed to nickel as nickel sulfate hexahydrate, nickel oxide, or nickel subsulfide for 12 days (6-hour/day) over 16 days (NTP 1996a, 1996b, 1996c). The maximum concentrations that did not result in deaths or changes in brain histology were 3.1, 23.6, and 7.33 mg Ni/m^3 in Fischer-344 rats for nickel sulfate hexahydrate, nickel oxide, and nickel subsulfide, respectively, and 0.7, 23.6, and 3.65 mg/m^3 in B6C3F1 mice for nickel sulfate hexahydrate, nickel oxide, and nickel subsulfide, respectively.

In intermediate-duration studies, no histological alterations are observed in the whole brains of Fischer-344 rats and B6C3F1 mice exposed to 0.44, 7.9, or 1.83 mg Ni/m^3 as nickel sulfate hexahydrate, nickel oxide, or nickel subsulfide, respectively, 6 hours/day, 5 days/week for 13 weeks (NTP 1996a, 1996b, 1996c). Exposure for 6 hours/day for 16 days to cobalt sulfate heptahydrate in male Long-Evans rats at 0.635 mg Ni/m^3 resulted in histological changes including decreased bipolar receptor cells and atrophy in the septal olfactory epithelium (Evans et al. 1995). However, no changes of olfactory function were noted following completion of behavioral studies for olfactory absolute threshold (odor detection) and discrimination. Thinning (atrophy) of the epithelium appeared normal after 12 days of recovery, and carnosine, a neurochemical marker, was reduced in the olfactory epithelium only at 12 days of exposure. Carnosine levels in the olfactory bulb were reduced up to the 12^{th} day of exposure and returned to control levels by the 16^{th} exposure day. Study authors attributed the recovery of carnosine levels during the exposure period to a defensive response against continued exposure (Evans et al. 1995). In Fischer-344 rats exposed to nickel sulfide at 0.63 mg Ni/m^3 for 78 weeks (6 hours/day, 5 days/week), histological changes were not observed in the brain (Ottolenghi et al. 1975). Chronic-duration exposure of Fischer-344 rats to nickel oxide, nickel subsulfide, or nickel sulfate hexahydrate at concentrations up to 2, 0.73, or 0.11 mg Ni/m^3, respectively, or exposure of B6C3F1 mice to nickel oxide, nickel subsulfide, or nickel sulfate hexahydrate at concentrations up to 3.9, 0.88, or 0.22 mg Ni/m^3, respectively, did not result in microscopic changes in the whole brain (NTP 1996a, 1996b, 1996c).

Oral

Neurological effects of giddiness and weariness were observed among 20 of 32 workers who drank water during one work shift from a water fountain contaminated with nickel sulfate, nickel chloride, and boric acid (Sunderman et al. 1988). It was estimated that the workers were exposed to between 7.1–35.7 mg Ni/kg. Seven workers reported giddiness and six workers reported weariness within hours of the exposure. The contribution of boric acid to these effects is not known.

In a study designed to determine the absorption and elimination of nickel in humans, one male developed left homonymous hemianopsia (loss of sight in the same corresponding two left halves of the visual fields

of both eyes) 7 hours after ingesting a single dose of 0.05 mg Ni/kg as nickel sulfate in drinking water. The condition lasted for 2 hours (Sunderman et al. 1989b). The appearance of the visual defect involving the same two left halves of the visual fields in both eyes occurred soon after the peak serum concentration of nickel was reached, leading the investigators to suspect a causal relationship between nickel exposure and the loss of sight/visual field defect. The doses given to other subjects were lowered to 0.018 and 0.012 mg Ni/kg with no adverse effects.

Hypoactivity and increased salivation were clinical signs of toxicity observed in rats exposed for 3 days to doses ≥27.91 mg Ni/kg/day as nickel sulfate hexahydrate (Oller and Erexson 2007). In a 90-day study, lethargy, ataxia, prostration, irregular breathing, and reduce body temperature were observed in rats treated by gavage with nickel chloride (American Biogenics Corporation 1988). These effects were observed frequently at 25 mg Ni/kg/day, a dose at which all rats died, and at lower incidences at 8.6 mg Ni/kg/day, a dose at which 6/52 rats died. At the lower dose, it is not clear if the adverse neurological effects were observed only in the animals that died. No signs of neurological dysfunction were observed at 1.2 mg/kg/day. Microscopic examinations of whole brains did not reveal any changes in the brains of dogs treated with nickel salts at doses ≤62.5 mg Ni/kg/day for 2 years (Ambrose et al. 1976; American Biogenics Corporation 1988). No nickel-exposure related changes in relative brain weight were recorded in rats exposed for 13 weeks to doses of up to 28.8 mg Ni/kg/day as nickel sulfate (Obone et al. 1999). Multi-generation studies by Springborn Laboratories (2000a, 2000b) did not find any exposure-related changes in the brain following gross necropsy examination nor any clinical signs that would indicate neurotoxicity. Springborn Laboratories (2000a) exposed 2 generations of rat parents to 0.22 to 2.2 mg Ni/kg/day for 16 to 18 weeks.

Dermal

No studies were identified that examined adverse neurological effects in humans or animals after dermal exposure to nickel.

2.16 REPRODUCTIVE

Inhalation

An increase in the rate of spontaneous abortions (15.9%) was reported among a group of 356 women who worked in a nickel hydrometallurgy refining plant in the Arctic region of Russia as compared to the rate (8.5%) in 342 local female construction workers (Chashschin et al. 1994). Exposure concentrations were 0.08–0.196 mg Ni/m^3, primarily as nickel sulfate, and nickel concentrations in the urine of nickel workers were 3.2– 22.6 µg/L. Nickel levels in the urine of persons not occupationally exposed are generally <0.1

to 13.3 µg/L (Sunderman 1993). The investigators noted that the nickel-exposed women manually lifted heavy nickel anodes and that they may have experienced heat stress. These confounders, plus the lack of information on the selection of control group subjects, possible acute exposure to high concentrations of chlorine, and the lack of adequate control of possible confounding variables such as smoking habits, use of alcohol, and intercurrent disease, preclude establishing a causative relationship between nickel exposure and reproductive toxicity from this study. Several epidemiological studies examined the association between maternal occupational exposure to water soluble nickel at the start of pregnancy and the risk of varying fetal outcomes among a population living near a large complex of nickel, copper, and cobalt refineries operating in the Kola Peninsula (Vaktskjold 2006, 2007, 2008a, 2008b). Maternal occupation and birth outcomes were obtained from the Kola Birth Registry and used to categorize nickel exposure based on job (Vaktskjold 2006, 2007, 2008a). Exposure did not affect the risk of birthing a small for gestation age newborn (Vaktskjold et al. 2007), delivering a newborn with a genital malformation (Vaktskjold et al. 2006), or delivery of a newborn with musculoskeletal defects (Vaktskjold et al. 2008a). The adjusted odds ratio for per unit increase in maternal occupational exposure to water soluble nickel and birthing a small-for-gestation age (SGA) newborn is 0.84 (95% CI: 0.75-0.93) (Vaktskjold et al. 2007). The adjusted odds ratio for nickel-exposed women delivering a newborn with a genital malformation is 0.81 (95% CI: 0.52–1.26), and that for an undescended testicle is 0.76 (95% CI 0.40–1.47) (Vaktskjold et al. 2006). The adjusted odds ratio for per unit increases in maternal nickel exposure category and musculoskeletal defects is 0.96 (95% CI: 0.76–1.21) (Vaktskjold et al. 2008a). In a case-control study of the same population, workers of facilities within and outside of the refinery complex self-reported pregnancy outcomes and employment history (Vaktskjold et al. 2008b). There was no significant association between maternal occupational exposure to water soluble nickel in early pregnancy and the risk of spontaneous abortion; the adjusted odds ratio is 1.14 (95% CI: 0.95 – 1.37) (Vaktskjold et al. 2008b).

A cross-sectional study found nickel concentration in local air to be associated with decreased sperm concentration in men whose partners underwent assisted reproductive technology procedures (Huang et al. 2019). Study authors used $PM_{2.5}$ data from 2 monitoring stations from dates right before and during sperm sample collection, and the average nickel exposure was 2.72 ng/m^3. However, this study had severe limitations as most subjects only had one semen sample collected while others had up to 9 collections, additionally air monitoring data is not indicative of the true exposure, and there was limited consideration of exposure to other pollutants (Huang et al. 2019).

No reproductive effects were observed in male Fischer-344 rats exposed at 23.6, 7.33, and 12.2 mg Ni/m^3, and in B6C3F1 mice exposed at 23.6, 3.65 and 1.4 mg Ni/m^3 for 12-day exposure (6 hours/day) to nickel oxide, nickel subsulfide and nickel sulfate hexahydrate, respectively (NTP 1996a, 1996b, 1996c).

In intermediate-duration studies, sperm concentration was decreased by 21% in Fischer-344 rats exposed to nickel oxide at 7.9 mg Ni/m^3, with no effects at 3.9 mg/m^3 (NTP 1996a). No effects on sperm motility, morphology, or concentration were observed in Fischer-344 rats and B6C3F1 mice exposed to nickel subsulfide or nickel sulfate at concentrations up to 1.83 and 0.44 mg Ni/m^3, respectively, or in mice exposed to nickel oxide, nickel subsulfide, or nickel sulfate hexahydrate at concentrations up to 7.9, 1.83, or 0.44 mg Ni/m^3, respectively (NTP 1996a, 1996b, 1996c). Histological changes in the testes were not observed. No effect on the length of the estrous cycle was noted in mice or rats exposed to nickel sulfate hexahydrate at ≤0.44 mg Ni/m^3, nickel oxide at ≤7.9 mg Ni/m^3, or nickel subsulfide at ≤1.83 mg Ni/m^3 6 hours/day, 5 days/week, for 13 weeks (NTP 1996a, 1996b, 1996c).

Chronic-duration exposure of Fischer-344 rats and B6C3F1 mice to nickel oxide, nickel subsulfide, or nickel sulfate hexahydrate at concentrations up to 2, 0.73, or 0.11 mg Ni/m^3, respectively, and exposure of mice to nickel oxide, nickel subsulfide, or nickel sulfate hexahydrate at concentrations up to 3.9, 0.88, or 0.22 mg Ni/m^3, respectively, did not result in microscopic changes in the reproductive organs (NTP 1996a, 1996b, 1996c).

Oral

No studies were identified that examined reproductive effects in humans after oral exposure to nickel.

Several studies have examined the reproductive toxicity of nickel following oral exposure to rats, mice, or dogs. The studies have found conflicting results, with some studies identifying LOAELs for serious health effects and others identifying NOAELs at very similar dose levels. Pandey et al. (1999) reported an accumulation of nickel (in descending order of concentration) in the epididymis, testes, seminal vesicles, and prostate gland in Swiss mice orally exposed to nickel sulfate for 35 days. The accumulation of nickel in male reproductive tissues resulted in histological damage in the epididymis and seminal vesicles and sperm damage. Regressed epithelium and vacuolated cells were observed in the epididymis of mice administered 1.1 mg Ni/kg as nickel sulfate via gavage 5 days/week for 35 day s (Pandey et al. 1999). In the seminiferous tubules, the damage consisted of atrophy of centrally located tubules and disturbed spermatogenesis in mice administered 1.1 mg Ni/kg as nickel sulfate (5 days/week) (Pandey et al. 1999). The significance of these findings is not known because the incidence data and statistical analysis were not reported. Käkelä et al. (1999) reported a statistically significant decrease in seminiferous tubule diameter in Wistar rats exposed to 3.6 mg Ni/kg/day as nickel chloride in drinking water for 28 or 42

days. A significant decrease in basal spermatogonia was also observed in the rats exposed for 28 days, but not in the rats exposed for 42 days. Although it was not discussed in the report, the final body weights of males exposed for 28 days appear to be lower than control body weights; this may contribute to the histological findings in the maturing rats (Rehm et al. 2008). Other studies have not found histological alterations in male or female reproductive tissues in rats administered up to 25 mg Ni/kg/day as nickel chloride for 91 days (American Biogenics Corporation 1988), rats exposed to 28.8 mg Ni/kg/day as nickel sulfate in drinking water for 90 days (Obone et al. 1999), rats exposed to 2.2 mg Ni/kg/day as nickel sulfate administered via gavage for 18 weeks (Springborn Laboratories 2000a), or dogs exposed to 62.5 mg Ni/kg/day as nickel sulfate in the diet for 2 years (Ambrose et al. 1976).

Significant decreases in sperm count and sperm motility and sperm abnormalities (banana and detached head; acrosome up, down, or missing; curved neck and curved, bent, round, loop, and folded tail) were observed in mice administered ≥2.2 mg Ni/kg as nickel sulfate (decreased sperm count significant at 4.5 mg Ni/kg) or 2.5 mg Ni/kg as nickel chloride 5 days/week for 35 days (Pandey and Srivastava 2000); no sperm effects were observed at 1.1 or 1.2 mg Ni/kg as nickel sulfate or nickel chloride, respectively. Although the route of administration was not reported, it is assumed that the nickel chloride and nickel sulfate were administered via gavage. The investigators reported a dose-related decrease in body weight gain and decreases in absolute and relative testes, epididymis, seminal vesicle, and prostate gland weights at the two highest dose levels (2.2 and 4.5 mg Ni/kg as nickel sulfate and 2.5 and 4.9 mg Ni/kg as nickel chloride). Similarly, Pandey et al. (1999) reported decreases in sperm count and motility in mice administered 2.2 mg Ni/kg as nickel sulfate, 5 days/week for 35 days; an increase in sperm abnormalities was also observed at 1.1 mg Ni/kg. Although Pandey et al. (1999) did not report alterations in body weight gain, significant decreases in testes, epididymis, seminal vesicle, and prostate gland weights were observed. In both studies by Pandey et al., there were no significant alterations in the occurrence of a particular sperm abnormality; the total number of abnormalities was increased. Toman et al. (2012) did not observe any exposure-related changes in relative testis weight following 3-12 weeks of exposure to 4.53 mg Ni/kg/day as nickel chloride, however significant changes were observed in the testis upon histological examination. Study authors observed signs of degeneration of seminiferous epithelium and empty spaces in the epithelium indicating spermatogenesis disruption (Toman et al. 2012). Sobti and Gill (1989) reported increases in sperm head abnormalities in mice receiving a single gavage dose of 23, 28, or 43 mg/kg as nickel nitrate, nickel sulfate, or nickel chloride, respectively; it should be noted that this study was poorly reported and no information on number of animals tested was given. No alterations in sperm count, concentration, motility, or morphology were observed in the F0 or F1 rats administered 2.2 mg Ni/kg/day as nickel sulfate via gavage for 18 weeks (Springborn Laboratories 2000a).

In addition to the histological alterations and sperm alterations, alterations in fertility were observed in some studies, but not in all studies. Male-only exposure or male and female exposure to 3.6 mg Ni/kg/day as nickel chloride in drinking water resulted in decreased fertility in rats exposed for 28 days prior to mating (Käkelä et al. 1999). However, male rats exposed to 3.6 mg Ni/kg/day for 42 days prior to mating with unexposed females resulted in a small decrease in fertility (83 versus 100%) (Käkelä et al. 1999); suggesting regeneration of damaged tissues. In a single generation study in which rats were administered 6.7 mg Ni/kg/day as nickel sulfate hexahydrate via gavage for 2-weeks prior to mating, during mating, and during gestation, post-implantation loss was 475% greater than in controls (Springborn Laboratories 2000b). The severity of post-implantation loss appeared dose related as the mean incidence increased with doses up to 16.7 mg Ni/kg/day and the loss at 2.2 and 4.5 mg Ni/kg/day were not statistically different from the mean (Springborn Laboratories 2000b). In a 3-generation study in rats where the F0 and F1 generations were each exposed for 11 weeks, the F1 generation had a significantly higher number of stillbirths compared to controls at the lowest dose tested of 22.5 mg Ni/kg/day (Ambrose et al. 1976). These effects were not observed in the F0 generation exposed to the same doses.

Female-only exposure to concentrations as high as 13 mg/kg/day as nickel chloride in drinking water did not adversely affect fertility in rats (Käkelä et al. 1999). Interpretation of this study is limited by the small number of animals tested (six/gender/group) and the limited reporting of the results. No adverse effects on fertility were observed in a multigeneration study in which male and female rats exposed to doses as high as 55 mg Ni/kg/day as nickel chloride in drinking water for 11 weeks prior to mating (RTI 1988a, 1988b), in a single generation study in which rats were administered 16.7 mg Ni/kg/day as nickel sulfate via gavage for 2-weeks prior to mating, during mating, and during gestation (Springborn Laboratories 2000b), in a two-generation study involving gavage administration of up to 2.2 mg Ni/kg/day for 10 weeks prior to mating, during mating, gestation, and lactation (Springborn Laboratories 2000a), or in a multi-litter study in which female rats were exposed to doses as high as 31.6 mg Ni/kg/day (Smith et al. 1993).

Several acute-duration studies where pregnant mice were exposed to doses ranging from 10.29 to 41.19 mg Ni/kg/day as nickel chloride reported exposure-related reductions in fertility (Saini et al. 2013, 2014a, 2014b). Exposure to ≥11.38 mg Ni/kg/day on gestation days 6 to 13 resulted in increased post-implantation death and fetal resorption (Saini et al. 2013). Similarly, exposure to ≥11.35 mg Ni/kg/day on gestation days 0 to 5 resulted in reduced number of implantation sites and number of live fetuses per dam (Saini et al. 2014a). Lower doses were not tested in either of these studies therefore a NOAEL for these effects was not reported. Saini et al. (2014b) exposed mice to 10.29 to 41.19 mg Ni/kg/day on either gestation days 0 to 5, 6 to 13, or 14 to 18. Exposure on gestation days 0 to 5 resulted in reduced gestation

index compared to controls and fetal loss appeared to increase in severity with dose. No effects on reproduction were seen in mice exposed to doses ≤ 20.59 mg Ni/kg/day on gestation days 6 to 13, or 14 to 18. However, at the highest dose, litter size per dam was significantly less than controls (Saini et al. 2014b).

Dermal

No studies were identified that examined adverse reproductive effects in humans after dermal exposure to nickel.

Tubular degeneration of the testes was observed in rats treated dermally with nickel sulfate at 60 mg Ni/kg/day for 30 days (Mathur et al. 1977). No effects were found at 40 mg Ni/kg/day after 30 days or at doses of ≤100 mg Ni/kg/day after 15 days of treatment. In this study, there was no indication that the rats were prevented from licking the nickel sulfate from the skin; therefore, these effects could have resulted from oral exposure.

2.17 DEVELOPMENTAL

Inhalation

Several studies have reported developmental effects in offspring of adults exposed to nickel in occupational settings. Chashschin et al. (1994) reported an increase in the incidence of structural malformations (16.9%) in the offspring of female nickel hydrometallurgy refining plant workers as compared to the incidence (5.8%) in female construction workers. Although the specific structural malformations found were not stated, the investigators note that relative risks were 2.9 for all defects, 6.1 for cardiovascular system defects, and 1.9 for musculoskeletal defects. Exposure concentrations were 0.08–0.196 mg Ni/m^3, primarily as nickel sulfate, and nickel concentrations in the urine were 3.2–22.6 µg/L. Nickel levels in the urine of persons not occupationally exposed are generally <0.1 to 13.3 µg/L (Sunderman 1993). A number of possible confounders include heavy lifting, possible heat stress, lack of information on the selection of control group subjects, possible acute exposure to high concentrations of chlorine, and the lack of adequate control of possible confounding variables such as smoking habits, use of alcohol, and intercurrent disease, preclude establishing a causative relationship between nickel exposure and developmental toxicity from this study. A separate study of female refinery workers exposed to nickel found that there was a slight but non-significant association between maternal exposure to nickel and musculoskeletal defects at birth (adjusted OR=0.96; 0.76-1.21) (Arild et al. 2008). Authors noted that the study examined the risk of delivering a newborn with defects for women working in nickel-exposed areas, and not the fetal risk for these defects. Nickel exposure was determined by air sampling

and urine measurements obtained from facility records on air sampling and medical history, respectively, thus there is uncertainty on the concentrations to which workers were exposed (Arild et al. 2008).

Population level studies have indicated associations between exposure to nickel in ambient air and low birth weight in offspring. A cohort study in New England found higher levels of Ni $PM_{2.5}$ were associated with lower birth weight, and nickel exposure during pregnancy resulted in a mean birthweight decrease of 7 grams, and an 11% increase in risk of small-at-term birth (Bell et al. 2010). This study also examined differences between race and found that infants from African American mothers had a 12 gram decrease in birth weight per interquartile range while infants from white mothers had a 6 gram decrease in birthweight per interquartile range (Bell et al. 2010). Another study examined children born from 2000 to 2007 from the U.S. northeast and mid-Atlantic and authors reported a 5.7% risk increase of low birthweight per interquartile range of $PM_{2.5}$ nickel (Ebisu and Bell 2012). The mean gestational exposure to nickel across all locations was 0.006 µg/m^3. Additionally, the relative risk of low birthweight with an interquartile increase in $PM_{2.5}$ nickel was 10.2% lower among infants of African American mothers compared to white mothers (Ebisu and Bell 2012). Similarly, a European cohort of children born between 1994 and 2008 showed an increased risk of low birthweight with increased nickel $PM_{2.5}$ concentrations (Pedersen et al. 2016). This same study reported an increased risk of reduced mean head circumference with increasing nickel $PM_{2.5}$ and PM_{10} levels.

A decrease in fetal body weight was observed in the offspring of Wistar rats exposed to 1.6 mg Ni/m^3 as nickel oxide 23.6 hours/day on gestation days 1–21 (Weischer et al. 1980). No effect on fetal body weight was observed at 0.8 mg Ni/m^3, although decreased maternal body weight gain was observed at this concentration. No effects on the number of fetuses or on the weight of the placenta were observed (Weischer et al. 1980).

Oral

No studies were identified that examined developmental effects in humans after oral exposure to nickel.

The available animal data on developmental toxicity provide suggestive evidence that the developing fetus and neonates are sensitive targets of nickel toxicity. The most reported endpoint was fetal loss and decreased survival observed in the rat and mouse offspring in studies involving male-only exposure, female-only exposure, and combined male and female exposure in single generation, multi-litter, and multigeneration studies. The developmental effects were often reported at maternally toxic doses. Other developmental endpoints that have been examined include body weights, gross necropsy for abnormalities, and neurodevelopmental toxicity.

Male-only exposure to 3.6 mg Ni/kg/day as nickel chloride in drinking water for 28 days resulted in decreases in the number of pups born alive (2.7/dam versus 10.2/dam in controls), the number of pups surviving until postnatal day 4 (56% versus 100% in controls), and litter size at postnatal day 21 (1.3 pups versus 9.2 pups in controls) (Käkelä et al. 1999). However, when the male rats were exposed to 3.6 Ni/kg/day for 42 days, no significant alterations in pup viability or survival were observed (Käkelä et al. 1999). A NOAEL was not identified in this study.

Several studies examined female-only exposure to nickel (Berman and Rehnberg 1983; Käkelä et al. 1999; Smith et al. 1993). An increase in spontaneous abortions was observed in female mice exposed to 160 mg Ni/kg/day as nickel chloride in drinking water on gestational days 2–17 (Berman and Rehnberg 1983); no effects were observed at 80 mg Ni/kg/day. In contrast, no effects on the average number of neonates per litter were observed when mouse dams were treated by gavage on gestation days 8–12 with 90.6 mg Ni/kg/day as nickel chloride (a dose that resulted in a significant decrease in maternal body weight) (Seidenberg et al. 1986). Exposure of rats to 13 mg Ni/kg/day as nickel chloride in drinking water for 14 days prior to mating, during mating, gestation, and lactation resulted in a decreased pup survival from birth to postnatal day 4 (87 versus 100% in controls) and from postnatal day 4 to 21 (52 versus 90% in controls) (Käkelä et al. 1999); no significant effects were observed at 4.0 mg Ni/kg/day. Pup mortality was also observed in a multi-litter study in which rats were exposed to 0, 1.3 , 6.8, or 31.6 mg Ni/kg/day as nickel chloride in drinking water for 11 weeks prior to breeding and during two successive gestation and lactation periods (Smith et al. 1993). In the first litter, the percentages of dead pups per litter at postnatal day 1 were 1.7, 3.1, 0, and 13.2% in rats exposed to 0,1.3, 6.8, or 31.6 mg Ni/kg/day, respectively, (statistically significant at the high dose only); no significant alterations were observed in the number of dead pups at postnatal day 21. In the second litter, the number of litters with dead pups at birth (2, 7, 6, and 10%; statistically significant at high dose only), the percentages of dead pups per litter at postnatal day 1 (1.0, 4.3, 4.6, and 8.8%; statistically significant at all three dose levels), and the percentage of dead pups at postnatal day 21 (12.5, 13.4, 19.4, and 29.2%; significant at high dose only) were increased in rats exposed to 0, 1.3, 6.8, or 31.6 mg Ni/kg/day, respectively.

Offspring mortality was also observed in four studies involving combined male and female exposure (Ambrose et al. 1976; Käkelä et al. 1999; RTI 1988a, 1988b; Springborn Laboratories 2000b). Exposure of rats to 3.6–4.0 mg Ni/kg/day as nickel chloride in drinking water for 28 days prior to mating, during mating, gestation, and lactation adversely affected the litter size at postnatal day 21 (2.7/dam versus 9.2/dam in controls) and pup survival from postnatal day 4 to 21 (44 versus 90% in controls) (Käkelä et al. 1999); a NOAEL was not identified. Significant increases in post-implantation losses were observed in the offspring of rats administered 6.7 mg Ni/kg/day as nickel sulfate via gavage for 14 days prior to

mating, during mating, and gestation (Springborn Laboratories 2000b); at 16.7 mg Ni/kg/day, an increased number of dead pups at lactation day 0 and a decreased mean litter size were observed. This study identified a NOAEL of 4.5 mg Ni/kg/day. In a multigeneration study (Ambrose et al. 1976) involving exposure of rats to 0, 22.5, 45, or 90 mg Ni/kg/day as nickel chloride in the diet for 11 weeks prior to mating, during mating, gestation, and lactation, a dose-related increase in the number of stillborn pups was observed. An independent statistical analysis of the data using the Fisher Exact Test found significant increases in the total number pups born dead at 22.5 mg Ni/kg/day and higher for the F1a generation, 45 and 90 mg Ni/kg/day for the F1b generation, 90 mg Ni/kg/day for the F2a generation, 22.5 mg Ni/kg/day for the F2b generation, and 45 and 90 mg Ni/kg/day for the F3b generation. The study authors noted that the number of offspring (dead and alive) was progressively less with increasing nickel levels above 45 mg/kg/day (10.3, 10.6, 9.8, and 9.0 for 0, 22.5, 45, and 90 mg/kg/day, respectively); the number of offspring weaned per litter was also decreased with increasing nickel levels (8.1, 7.2, 6.8, and 6.4 for 0, 22.5, 45, and 90 mg/kg/day, respectively). The third study (RTI 1988a, 1988b) is a two-generation study in which the P0 generation was exposed to nickel chloride in drinking water for 11 weeks before mating and during gestation and lactation, and the F1b generation animals were mated to produce the F2 generations. A reduction in live litter size was observed in the F1a, F1b, and F2a offspring of rats exposed to 55 mg Ni/kg/day. Increases in mortality were also observed in the F1b rats on postnatal days 22 through 42; these increases were statistically significant in males at 30 and 55 m g Ni/kg/day and in females at 55 mg Ni/kg/day. No adverse developmental effects, including no effect on litter size, were observed in the cesarean delivered F2b rats, suggesting that the nickel-induced decrease in live litter size occurred postnatally. No alterations in offspring mortality or survival were observed in a two-generation study in which rats were administered up to 2.2 mg Ni/kg/day as nickel sulfate via gavage for approximately 18 weeks (Springborn Laboratories 2000a).

Several acute-duration studies in pregnant mice where reproductive changes were observed also reported development abnormalities in offspring. Maternal exposure to ≥11.38 mg Ni/kg/day on gestation days 6 to 13 resulted increased incidence of skeletal anomalies including reduced or fused sternebrae, absence or gap between the ribs, and reduced ossification, and a 5% incidence of microphthalmia (born with small eyes resulting in vision loss or blindness) (Saini et al. 2013). Maternal exposure to ≥11.35 mg Ni/kg/day on gestation days 0 to 5 also resulted in an increased incidence of skeletal anomalies that increased with dose (Saini et al. 2014a). The incidence of skeletal anomalies and significance of reduced body weight compared to controls increased with dose. Saini et al. (2014b) exposed pregnant mice to 10.29 to 41.19 mg Ni/kg/day on either gestation days 0 to 5, 6 to 13, or 14 to 18. The lowest LOAEL for developmental effects in this study was among mice exposed on gestation days 6 to 13 where offspring body weight was significantly lower at birth than controls at 10.29 mg Ni/kg/day and >9% fetal mortality was reported at

higher doses. A 12% increase in fetal mortality was reported at 41.19 mg Ni/kg/day from exposure on gestation days 0 to 5, and an 11% increase at 20.59 mg Ni/kg/day from exposure on gestation days 14 to 18 (Saini et al. 2014b). El Sekily et al. (2020) similarly exposed pregnant female mice to 10.29 to 41.08 mg Ni/kg/day as nickel chloride on gestation days 6 to 13 and reported a significant increase in fetal resorption sites at all doses and a significant number of stillborn fetuses at 41.08 mg Ni/kg/day. Skeletal abnormalities are reported in offspring exposed to all doses, including incomplete ossification of the skull, vertebrae, ribs, and limbs, and unossified carpals, metacarpals, tarsals, metatarsals, and phalanges (El Sekily et al. 2020).

Decreases in pup body weights were reported in the offspring of rats exposed to 90 mg Ni/kg/day (Ambrose et al. 1976), 30, and 55 mg Ni/kg/day (RTI 1988a, 1988b). Neither the Ambrose et al. (1976) nor the RTI (1988a, 1988b) multigeneration studies found a significant increase in the incidence of gross abnormalities in the surviving offspring of rats exposed to nickel. Käkelä et al. (1999) noted that the pups that died during lactation were runts (smaller or weaker animals in a litter): the heads were disproportionately large, and the posteriors of the bodies were underdeveloped. No effect on locomotor activity was observed following a figure 8 maze test in the offspring of mice treated by gavage at 45.3 mg Ni/kg/day as nickel chloride on gestation days 8–12 (Gray et al. 1986).

In summary, these data provide suggestive evidence that exposure to nickel prior to mating and during gestation and lactation results in decreased offspring survival (Ambrose et al. 1976; Käkelä et al. 1999; RTI 1988a, 1988b; Smith et al. 1993). Decreased survival was also observed in the offspring of male rats exposed prior to mating to unexposed females (Käkelä et al. 1999) and increased spontaneous abortions were observed following gestation-only exposure of mice (Berman and Rehnberg 1983). Interpretation of these data is complicated by the maternal toxicity, in particular, a decrease in maternal body weight gain, which was also observed at these dose levels (Ambrose et al. 1976; Käkelä et al. 1999; RTI 1988a, 1988b; Smith et al. 1993). Decreases in food and water intake have also been observed (RTI 1988a, 1988b; Smith et al. 1993).

Dermal

No studies were identified that examined adverse developmental effects in humans or animals after dermal exposure to nickel.

2.18 OTHER NONCANCER

Inhalation

This section on other noncancer effects includes discussion on metabolic effects, including discussion on serum glucose levels. Urinary and serum glucose levels may also be discussed in other sections of Chapter 2 as relevant to the discussed health effects. No studies were identified that examined noncancer effects in humans after inhalation exposure to nickel.

Significant increases (13%) in serum glucose levels were observed in male Wistar rats continuously exposed to 0.385 mg Ni/m^3 as nickel oxide for 28 days (23.6 hours/day) (Weischer et al. 1980). In females rats continuously (23.6 hours/day) exposed to nickel oxide, a 19% decrease in serum glucose levels was observed at 0.8 mg Ni/m^3 (Weischer et al. 1980). These data suggest that there may be a sex difference.

In male and female Wistar rats exposed to 0.4 mg Ni/m^3 metallic metal for 104 weeks (5 days/week, 6 hours/day), reduced mean food consumption was exposure-related (Oller et al. 2008). For males this occurred from week 58 to 104, and for females, from weeks 66 to 87.

Oral

No studies were identified that examined other noncancer effects in humans after oral exposure to nickel.

Two studies reported significant alterations in serum glucose levels in rats exposed to nickel chloride. A significant decrease in blood glucose levels was observed in female rats administered 8.6 mg Ni/kg/day via gavage for 91 days (American Biogenics Corporation 1988). In contrast, Weischer et al. (1980) reported a significant increase in blood glucose levels in male rats administered 0.23 mg Ni/kg/day via drinking water for 28 days. In both studies, significant decreases in body weight gain (20% and higher) were also observed at the same dose effect levels. Thus, it is difficult to assess whether this is a direct effect of nickel or secondary to the effect on body weight.

Dermal

Blood glucose levels were significantly increased in guinea pigs treated with 100 mg Ni/kg as nickel sulfate placed on skin of the back for 15 or 30 days (Mathur and Gupta 1994). There was no indication that the animals were prevented from licking the nickel from the skin; therefore, these effects could have resulted from oral exposure.

2.19 CANCER

Inhalation

The carcinogenic potential of nickel has been examined in many population and occupational studies. Associations between breast cancer and air exposure was analyzed in several studies using data from the EPA's Toxics Release Inventory (TRI) to estimate county-level exposures to nickel (Coyle et al. 2005; Kresovich et al. 2019; White et al. 2019). Using county-level estimates of nickel in air, Coyle et al. (2005) concluded that age-adjusted breast cancer rates were not associated with nickel release in women over 50 years of age. In a prospective study, White et al. (2019) followed 50,884 cancer-free women and did not find nickel concentrations in air to be associated with a higher risk of breast cancer. Kresovich et al. (2019) did not find that nickel ambient air exposure in Chicago, calculated by census-tract level from TRI data, increased the odds of developing ER/PR-negative breast tumors.

Several population-level studies have examined associations between nickel in ambient air and different types of cancers. A study in California analyzing cases of children diagnosed with retinoblastoma between 1990 and 2007 found a significantly increased risk of diagnosis associated with higher nickel exposures during pregnancy (Heck et al. 2015). A non-significant increased risk was also reported for exposure to nickel during a child's first year of life. Air concentration data was collected from several monitors during the same years of diagnosis and calculated a mean nickel air concentration of 5.08 ng/m^3 (Heck et al. 2015). A different study of children in California by Whitehead et al. (2015) specifically looked at exposure to nickel from carpet dust and found no significant association with development of acute lymphoblastic leukemia.

Two studies have found associations between increased risk of lung cancer and nickel exposures. Luo et al. (2011) analyzed TRI county-level data on on-site releases to air, water, surface land, and surface injection, and found an increased risk of lung cancer in counties with non-zero nickel releases. An analysis of 14 European cohort measured Ni $PM_{2.5}$ and PM_{10} in cohort areas from October 2008 to May 2011 and recorded a statistically significant association between risk of lung cancer among those who did not move away from the cohort area and PM_{10} nickel (Raaschou-Nielsen et al. 2016).

Several occupational studies have found statistically significant increases in the risk of nasal and/or lung cancer among nickel refinery workers generally employed between 1910 and 1985 at sulfidic nickel refineries, mines, and smelters (Andersen et al. 1996; Anttila et al. 1998; Chovil et al. 1981; Doll et al. 1977; Enterline and Marsh 1982; Grimsrud et al. 2003; Karjalainen et al. 1992; Magnus et al. 1982; Muir et al. 1994; Pedersen et al. 1973; Peto et al. 1984; Roberts et al. 1989b). Sorahan and Williams (2005) provided an update on a cohort of 812 workers at the Clydach nickel carbonyl refinery in South Wales,

employed from 1953 to 1992; this facility was previously studied by Doll et al. (1977) and Peto et al. (1984). Among all workers, the standardized mortality ratio (SMR) was non-significant for excess lung cancer however it was significant among workers employed for at least 5 years in feed handling and nickel extraction (Sorahan and Williams 2005). The same study did not find increased cancer mortality for other sites among this cohort. Study authors concluded that lung cancer deaths could not be linked to nickel exposure however since previous studies at this refinery have found an association, further study is warranted (Sorahan and Williams 2005). A study by Pavela et al. (2017) analyzed workers employed from 1967 to 2011 at a previously studied nickel refinery and smelter in Finland (Anttila et al. 1998; Karjalainen et al. 1992). This study confirmed that exposure to nickel compounds primarily contributed to excess risk of lung cancer in both men and women working at this facility, reporting standardized incidence ratios (SIRs) of 1.05 and 1.22, respectively (Pavela et al. 2017). Sunderman et al. (1989a) examined the histopathological diagnosis of 100 cases of sinonasal cancer and 259 cases of lung cancer among workers at three nickel refinery facilities. The primary sinonasal cancers were squamous cell carcinomas (48%), anaplastic and undifferentiated carcinomas (39%), and adenocarcinomas (6%). In an analysis of lung cancer, the cancers were primarily squamous cell carcinomas (67%), anaplastic, small cell, and oat cell carcinomas (15%), and adenocarcinomas (8%). The types of sinonasal and lung cancers were similar to those found in the general population, suggesting a lack of nickel-specific tumor types.

Two case-control studies of German male workers employed from 1988-1996 reported that among those welding regularly, high nickel exposure was associated with an increased risk of lung cancer when adjusting for exposure to welding fumes and hexavalent chromium (Pesch et al. 2019).

In contrast, most studies in other groups of nickel workers have not found significant increases in the risk of lung cancer among workers. This includes workers in mines (Shannon et al. 1984a; Shannon et al. 1991), hydrometallurgical refineries (Egedahl and Rice 1984; Egedahl et al. 2001; Egedahl et al. 1991), nickel alloy and stainless steel production facilities (Cornell 1984; Cornell and Landis 1984; Cox et al. 1981; Enterline and Marsh 1982; Jakobsson et al. 1997; Moulin et al. 1993; Sorahan 2004), stainless steel welders (Danielsen et al. 1996; Gérin et al. 1993; Hansen et al. 1996; Simonato et al. 1991), workers involved in nickel-chromium electroplating (Pang et al. 1996), workers of a barrier production facility (Cragle et al. 1984; Godbold and Tompkins 1979), or hard metal production workers (Marsh et al. 2017a; Marsh et al. 2017b). Although some studies of these workers did find significant increases in respiratory tract cancers (Becker 1999; Moulin et al. 1990), the increased risk was attributed to exposure to other carcinogenic agents, such as polycyclic aromatic hydrocarbons or asbestos. Redmond (1984) and Arena et al. (1998) reported significant increases in lung cancer risks among exposed nickel alloy production workers as compared to the general U.S. population. However, when the local population was used as the

comparison group, the increase in lung cancer risk was no longer statistically significant (Arena et al. 1998). In general, workers employed in these industries were exposed to lower levels of sulfidic or oxidic nickel than the nickel refinery workers who were primarily exposed to metallic nickel (Cragle et al. 1984; Godbold and Tompkins 1979) or soluble nickel (Pang et al. 1996). A broader population-based lung cancer case-control study in Europe did not find an increased risk from exposure to nickel dust or fumes in occupational settings (Mannetje et al. 2011).

Two studies found significant increases in the incidence of stomach cancer among nickel refinery workers (Anttila et al. 1998) and nickel platers (Pang et al. 1996). These data are insufficient to conclude whether the increases in stomach cancer risks are due to exposure to nickel, other agents, or chance. A meta-analysis of occupational exposure studies on pancreatic cancer (Ojajärvi et al. 2000) found a significant association between exposure to nickel and pancreatic cancer risk. However, the Ojajärvi et al. (2000) meta-analysis has been criticized (Seilkop 2001) for excluding a study of nickel mining and smelting workers (Shannon et al. 1991) and a study of nickel alloy production workers (Arena et al. 1998). The addition of these studies lowered the meta-analysis ratio from 1.9 (95% confidence interval 1.2–3.2) to 1.3 (95% confidence interval 0.9–1.9). A recent case-control study of pancreatic cancer patients from the Mayo Clinic did not find a significant relationship between self-reported nickel exposure in the work environment and pancreatic cancer risk (Antwi et al. 2015). A 7-country case-control study of glioma cases did not find that occupational exposure to nickel or welding fumes increased the risk of disease development, even when accounting for cumulative exposure (Parent et al. 2017). Additionally, two case-control studies of individuals with testicular germ cells tumors found that neither paternal or maternal occupational exposure to solvents and heavy metals including nickel increased the risk of tumors (Olsson et al. 2018; Togawa et al. 2016). Overall, there does not appear to be sufficient evidence that exposure to airborne nickel is associated with increased cancer risks outside of the respiratory tract.

Several animal studies have examined the carcinogenic potential of nickel subsulfide, nickel oxide, and nickel sulfate hexahydrate. Chronic-duration exposure to nickel subsulfide resulted in significant increases in lung tumors in two rat studies. Adenomas, adenocarcinomas, squamous cell carcinomas, and fibrosarcoma were observed in rats exposed to 0.63 mg Ni/m^3 as nickel sulfide for 78 weeks, 6 hours/day, 5 days/week (Ottolenghi et al. 1975). Similarly, significant increases in the combined incidences of alveolar/ bronchiolar adenoma or carcinoma were observed in male and female rats exposed to 0.11 or 0.73 mg Ni/m^3 as nickel subsulfide, 6 hours/day, 5 days/week for 2 years (NTP 1996b). In contrast, Wistar rats exposed to concentrations up to 1 mg Ni/m^3 as a nickel powder for 24 months, 6 hours/day, 5 days/weeks, did not show increased incidence of respiratory tract neoplasms, but other signs of lung toxicity were present (Oller et al. 2008). However, this same study found that the incidence of benign and

malignant adrenal gland pheochromocytoma in male rats and cortical adenoma/carcinomas in females were concentration-dependent to nickel metal exposure and increased tumor incidence was significant at 0.4 mg Ni/m^3 for both sexes (Oller et al. 2008). The study authors noted the incidence of cortical adenoma/carcinomas in females falls within historical ranges for control and cannot be definitely linked to the nickel exposure. Significant increases in the incidence of benign or malignant pheochromocytoma in the adrenal medulla were also observed in male rats at 0.11 or 0.73 mg Ni/m^3 and in females at 0.73 mg Ni/m^3 nickel subsulfide (NTP 1996b). In contrast to the findings in rats, no significant alterations in tumor incidences were observed in mice exposed to 0.44 or 0.88 mg Ni/m^3 as nickel subsulfide 6 hours/day, 5 days/week for 2 years (NTP 1996b) or in mice following weekly intratracheal injections of ≤0.8 mg Ni/m^3 as nickel subsulfide for ≤15 weeks, followed by observation for ≤27 months (Fisher et al. 1986; McNeill et al. 1990). Acute-duration (6 hours/day, 5 days/week, for 1 month) inhalation exposure to ≤6.3 mg Ni/m^3 as nickel oxide resulted in no significant increase in lung cancer in rats ≤20 months after exposure (Horie et al. 1985). However, significant increases in the incidence of alveolar/bronchiolar adenoma or carcinoma were observed in male and female rats exposed to 1 or 2 mg Ni/m^3 as nickel oxide 6 hours/day, 5 days/week for 2 years (NTP 1996c), but not in rats exposed to 0.5 mg Ni/m^3 or in mice exposed to 1, 2, or 3.9 mg Ni/m^3. Significant increases in the incidence of benign or malignant pheochromocytoma in the adrenal medulla were also observed in rats exposed to 3.9 mg Ni/m^3 (NTP 1996c). In contrast to the less soluble nickel compounds, chronic-duration (6 hours/day, 5 days/week for 2 years) exposure to nickel sulfate did not result in significant increases in neoplasms in rats or mice (NTP 1996a); the highest concentrations tested were 0.11 and 0.22 mg Ni/m^3, respectively.

The U.S. Department of Health and Human Services (NTP 2016) has determined that metallic nickel may reasonably be anticipated to be a human carcinogen and that nickel compounds are known to be human carcinogens. Similarly, IARC (IARC 1990b, 2021) classified metallic nickel in group 2B (possibly carcinogenic to humans) and nickel compounds in group 1 (carcinogenic to humans). EPA has classified nickel refinery dust and nickel subsulfide in Group A (human carcinogen) (IRIS 1987a, 1987b) and nickel carbonyl in Group B2 (probable human carcinogen) (IRIS 1987c). Other nickel compounds have not been classified by the EPA. Based on the occupational data, inhalation unit risk levels of $2.4x10^{-4}$ $(\mu g/m^3)^{-1}$ and $4.8x10^{-4}$ $(\mu g/m^3)^{-1}$ were derived for nickel refinery dust and nickel subsulfide, respectively (IRIS 1987a, 1987b). The risk levels range from $4x10^{-1}$ to $4x10^{-4}$ $\mu g/m^3$ for a risk ranging from $1x10^{-4}$ to $1x10^{-7}$, respectively, for nickel refinery dust (IRIS 1987a) and from $2x10^{-1}$ to $2x10^{-4}$ $\mu g/m^3$ for a risk ranging from $1x10^{-4}$ to $1x10^{-7}$, respectively, for nickel subsulfide (IRIS 1987b).

Nickel-induced alterations in gene expression may be mediated by activated transcription factors. Nickel has been shown to alter several transcription factors including hypoxia-inducible transcription factor

(HIF-1) and activated transcription factor (ATF-1) (Kasprzak et al. 2003). Nickel exposure is associated with accumulation of HIF-1 which is involved in the regulation of hypoxia-inducible genes involved in cell transformation, tumor promotion, and progression, angiogenesis, altered metabolism, and apoptosis (Salnikow et al. 2003). HIF-1α, one of the HIF-1 subunits, is over-expressed in both primary and metastatic tumors, and is induced in response to hypoxia and exposure to nickel (Li et al. 2004; Salnikow et al. 2000). Both soluble and insoluble nickel compounds have also been shown to induce Cap43 (also called NDRG1) gene expression, a tumor marker, which requires HIF-1α activation (Costa et al. 2003; Li et al. 2004; Salnikow et al. 2000, 2003). Nickel (II) via reactive oxygen species (ROS) can imitate cellular hypoxia without activating HIF-1 dependent genes (Salnikow et al 1994). The ability of nickel to activate HIF-1α transcription factors may be attributed to nickel's capacity to substitute iron (II) in oxygen transport and formation of non-functional hemoglobin (Das et al. 2019). Nickel-transformed rat and mice cells show that the induction of ATF-1 transcription factor down-regulates thrombospondin-1 (TSP-1) expression (Kasprzak et al. 2003; Salnikow et al. 1997). TSP-1 suppresses angiogenesis; thus, the suppression of TSP-1 stimulates tumor growth.

Oral

No studies were identified that examined cancer in humans after oral exposure to nickel. A few studies have found a correlation between nickel levels in local farm soils and increased incidences of different cancers however these studies are very limited as exposure scenarios to soils are not established and other factors and exposures cannot be fully considered in the analyses (Huang et al. 2013; Lee et al. 2016a; Su et al. 2010).

In lifetime drinking water studies in rats and mice, nickel acetate (0.6 mg Ni/kg/day for rats; 0.95 mg Ni/kg/day for mice) was found to be noncarcinogenic (Schroeder et al. 1964; Schroeder et al. 1974). The incidence of tumors was comparable to that observed in controls. Similarly, neoplastic and non-neoplastic findings in Fischer-344 rats exposed for 2 years to doses up to 11.16 mg Ni/kg/day were not related to nickel exposure and were similar to the control group (Heim et al. 2007).

Dermal

No studies were identified that examined cancer in humans or animals after dermal exposure to nickel.

2.20 GENOTOXICITY

A number of studies have examined the genotoxicity of nickel and nickel compounds; the results of these *in vivo* and *in vitro* tests are presented in Table 2-5 and Table 2-6, respectively. The available weight of evidence suggests that nickel does not alter the frequency of gene mutations in nonmammalian organisms

(Arlauskas et al. 1985; Biggart and Costa 1986; Green et al. 1976; Marzin and Phi 1985; Rasmuson 1985; Wong 1988), although some studies have found gene mutations (Iyehara Ogawa et al. 1994; Pikálek and Necásek 1983; Rodríguez-Arnaiz and Ramos 1986). Mixed results for gene mutations have been found in mammalian test systems. Increases in the frequency of gene mutations have been found at the HGPRT locus in Chinese hamster V79 cells exposed to nickel (Hartwig and Beyersmann 1989; Miyaki et al. 1979; Ohshima 2003). Two studies on V79 cells (Åkerlund et al. 2018; Buxton et al. 2020) and another in Chinese hamster ovary cells (Hsie et al. 1979) failed to find evidence of gene mutations at this locus. An increase in gene mutation frequency has also been found in Chinese hamster ovary AS52 cells (grp locus) (Fletcher et al. 1994), mouse lymphoma cells (Amacher and Paillet 1980; McGregor et al. 1988), and virus-infected mouse sarcoma cells (Biggart and Murphy 1988; Biggart et al. 1987). Gene mutation frequency was not affected in transgenic mouse and rat respiratory tissue following inhalation exposure to nickel subsulfide (Mayer et al. 1998). Dominant lethal mutations were not affected by intraperitoneal exposure of nickel acetate in mice (Deknudt and Léonard 1982). Nickel acetate exposure ranging from 0.5 mg/kg to 5 mg/kg was associated with increased frequency of dominant lethal mutations in germline cells of mice (Domshlak et al. 2005). Additionally, increased frequency of gene mutations was observed in pigment cells of first-generation mice at doses above 1.0 mg/kg (Domshlak et al. 2005). There is evidence to suggest that nickel is clastogenic and can damage DNA. Chromosome gaps or chromosome aberrations have been reported in several studies of lymphocytes from nickel refinery workers (Deng et al. 1988; Waksvik and Boysen 1982; Waksvik et al. 1984). Workers in a welding factory exposed to high concentrations of nickel (0.340-10.129 mg/m^3) showed significant increases in chromosomal aberrations relative to unexposed controls, though the controls were co-exposed to chromium and PAHs (Borská et al. 2003). *In vivo* studies show that intraperitoneal injection resulted in chromosomal aberrations in mouse bone marrow cells following nickel chloride exposure (Dhir et al. 1991; El-Habit and Abdel Moneim 2014), and in rat bone marrow and spermatogonial cells following nickel sulfate exposure (Mathur et al. 1978). *In vitro* assays have found chromosomal abnormalities using hamster cells (Conway and Costa 1989; Larramendy et al. 1981; Ohshima 2003; Sen and Costa 1986; Sen et al. 1987), mouse embryo cells (Clemens and Landolph 2003; Terpilowska and Siwicki 2018), human lymphocytes (Larramendy et al. 1981; Lechner et al. 1984), human bronchial epithelial cells (Holmes et al. 2013; Lechner et al. 1984), and human liver cancer cells (Terpilowska and Siwicki 2018). In a metaphase analysis of human lymphocytes from nickel-hypersensitized and nickel-unsensitized subjects, positive evidence of genotoxicity was observed (Arrouijal et al. 1992).

No alterations in the occurrence of sister chromatid exchange were observed in two studies of lymphocytes from nickel refinery workers (Waksvik and Boysen 1982; Waksvik et al. 1984), but another found that nickel workers had significantly higher levels of sister chromatid exchange than unexposed

controls (Deng et al. 1988). Increases were also found in *in vitro* assays of human lymphocytes (Andersen 1983; Arrouijal et al. 1992; Larramendy et al. 1981; M'Bemba-Meka et al. 2007; Saxholm et al. 1981; Wulf 1980) and hamster cells (Andersen 1983; Hartwig and Beyersmann 1989; Larramendy et al. 1981; Saxholm et al. 1981).

In vitro studies suggest that exposure to nickel leads to cell transformation in mammalian cells. Positive evidence for cell transformation has been observed in several types of hamster cells: Chinese hamster ovary cells (Conway and Costa 1989; Costa and Mollenhauer 1980; Costa et al. 1982), Chinese hamster embryo cells (DiPaolo and Casto 1979), Syrian hamster embryo cells (Conway and Costa 1989; Costa and Mollenhauer 1980; Costa et al. 1982), and baby kidney hamster cells (Hansen and Stern 1984). Cell transformation was also found in human foreskin (Biedermann and Landolph 1987) and mouse embryo cells (Clemens and Landolph 2003; Saxholm et al. 1981). Miura et al. (1989) observed cell transformation in mouse embryo cells exposed to nickel subsulfide, nickel monosulfide, and nickel oxide, but not in those exposed to nickel sulfate or nickel chloride.

Micronucleus formation was not affected in several studies of rat or mouse bone marrow cells following oral or intraperitoneal exposure (Covance Laboratories 2003; Deknudt and Léonard 1982; Morita et al. 1997). One study found increased micronuclei formation in bone marrow cells of mice exposed to nickel chloride via intraperitoneal injection (El-Habit and Abdel Moneim 2014). Exposed welders with a mean blood nickel concentration of approximately 5 μg/L had significantly higher frequency of micronuclei than controls, though it should be noted that co-exposures to chromium and lead occurred (Iarmarcovai et al. 2005). Increased micronuclei formation was observed in one *in vitro* study of human lymphocytes from nickel-unsensitized subjects, and the effect was dose-dependent and 50% greater than in nickel-sensitized subjects (Arrouijal et al. 1992). No evidence of increased micronuclei formation was found in several studies including an immortalized human bronchial epithelial cell line (BEAS-2B) (Gliga et al. 2020), human colon cancer cells (Kim and Seo 2011), and Chinese hamster V79 cells (Buxton et al. 2020; Nordin et al. 2018).

DNA damage has been observed in several *in vivo* studies in mice and rats. In mice exposed to single nose-only inhalation doses of nickel subsulfide, DNA damage in lung and nasal mucosal cells consisted of fragmentation (Mayer et al. 1998). Significant DNA damage was observed at all doses in bone marrow cells of mice given intraperitoneal injections of nickel chloride from 40 to 120 μmol/kg BW (El-Habit and Abdel Moneim 2014). Intraperitoneal administration for 2 weeks of 2 or 20 mg/kg also resulted in significant DNA fragmentation of peripheral blood mononuclear cells (Jia and Chen 2008). DNA damage was observed in leukocytes of mice orally exposed to nickel chloride at doses ranging from 3.4 to 108.8 mg/kg (Danadevi et al. 2004). Two studies observed significant increases in DNA double-strand breaks in

mouse sperm cells following intraperitoneal administration to either nickel sulfate or nickel chloride (Domshlak et al. 2005; Doreswamy et al. 2004). In isolated lung cells from rats exposed to concentrations ≤0.22 mg Ni/m^3 as nickel sulfate hexahydrate, DNA damage was not increased after 3 weeks but appeared to increase after 13 weeks (Oller et al. 2022). Exposure to nickel subsulfide showed DNA damage increased with exposure concentration regardless of duration (Oller et al. 2022). Evidence from *in vivo* studies in humans has been mixed. DNA oxidative damage was observed in nickel smelting workers and correlated with length of employment (Cheng et al. 2019). Workers with a mean blood nickel concentration around 5 μg/L had significant increases in DNA damage of lymphocytes relative to controls (Iarmarcovai et al. 2005). Oxidative DNA damage, as assessed by levels of plasma 8-hydroxyguanosine, was significantly associated with nickel in umbilical cord blood in pregnant women (Ni et al. 2014), nickel urine in smelting workers (Wu et al. 2015), and employment length in nickel smelting workers (Wu et al. 2015). In a study of U.S. factory workers, urine 8-hydroxyguanosine was also significantly associated with air concentrations of nickel (Kim et al. 2004). A study of orthodontic treatments containing nickel and chromium found evidence of DNA damage in buccal mucosa, but linear regression analyses indicated these effects were unrelated to nickel content (Hafez et al. 2011). In a study of Chinese men (n = 516), urine nickel (mean of 2.0 μg/L) was not associated with DNA damage in sperm cells (Wang et al. 2016a).

Two studies of prokaryotic organisms – one in *Bacillus subtilis* (Kanematsu et al. 1980) and one in *S. typhimurium* (Keyhani et al. 2006) – found no evidence of DNA damage upon exposure to nickel. Nickel significantly altered DNA replication rate in *E. coli* (Chin et al. 1994). One study of eukaryotic organisms was located, which found no evidence of reverse mutation in *Saccharomyces cerevisiae* after exposure to nickel (Singh 1984).

Most in vitro studies of nickel exposure have found positive evidence of DNA damage in mammalian cells. DNA damage was found in mouse fibroblast cells (Terpilowska and Siwicki 2018; Wang et al. 2016b) and rat kidney cells (Chen et al. 2010). DNA protein crosslink and/or single strand breaks have also been observed in Chinese hamster ovary cells (Hamilton-Koch et al. 1986; Patierno and Costa 1985) and V79 cells (Nordin et al. 2018). Several studies have noted DNA damage in human lymphocytes exposed to nickel (Chen et al. 2003; Rao et al. 2008; M'Bemba-Meka et al. 2005). DNA damage has also been observed in numerous types of epithelial cells following exposure to nickel: umbilical cord endothelial cells (Beck et al. 2014), alveolar epithelial cells (Di Pietro et al. 2009; Schwerdtle and Hartwig 2006), bronchial epithelial cells (Di Bucchianico et al. 2018; Castorina and Giunta 2014; Gliga et al. 2020), and human proximal tubule epithelial cells (Wang et al. 2012). DNA damage to fibroblasts has been found in dermal (Belliardo et al. 2018) and fetal (Qiao and Ma 2013) cell cultures. Additional

evidence of DNA damage are from *in vitro* studies of leukemic cells (Cavallo et al. 2003; Jia and Chen 2008), lymphoblastoid cells (Guillamet et al. 2008; Lou et al. 2013), colon cancer cells (Kim and Seo 2011, 2012), and liver cancer cells (Terpilowska and Siwicki 2018). In a study of HeLa cells, exposure to nickel adversely affected DNA replication (Chin et al. 1994). DNA single strand breaks and damage (as assessed using comet analysis) were not found in human diploid fibroblasts (Hamilton-Koch et al. 1986) or human gastric mucosal cells (Pool-Zobel et al. 1994), respectively.

Table 2-5. Genotoxicity of Nickel *In Vivo*

Species (test system)	Endpoint	Results	Reference	Compound
Drosophila melanogaster	Gene mutation	–	Rasmuson 1985	Nickel nitrate or chloride
D. melanogaster	Recessive lethal	+	Rodríguez-Arnaiz and Ramos 1986	Nickel sulfate
D. melanogaster	Gene mutation (wing spot test)	(+)	Iyehara Ogawa et al. 1994	Nickel chloride
Mammalian cells:				
Human lymphocytes	Chromosome gaps	+	Waksvik and Boysen 1982	Nickel oxide, nickel subsulfide
Human lymphocytes	Sister chromatid exchange	–	Waksvik and Boysen 1982	Nickel oxide, nickel subsulfide
Human lymphocytes	Chromosome aberrations	+	Waksvik et al. 1984	Nickel
Human lymphocytes	Sister chromatid exchange	–	Waksvik et al. 1984	Nickel
Human lymphocytes	Chromosome aberrations	+	Deng et al. 1988	Nickel
Human lymphocytes	Sister chromatid exchange	+	Deng et al. 1988	Nickel
Human lymphocytes	Chromosome aberrations	+	Borská et al. 2003	Nickel
Human lymphocytes	DNA damage	+	Iarmarcovai et al. 2005	Nickel
Human lymphocytes	Micronuclei formation	+	Iarmarcovai et al. 2005	Nickel
Human blood cells	Oxidative DNA damage	+	Cheng et al. 2019	Nickel
Human umbilical cord blood	Oxidative DNA damage	+	Ni et al. 2014	Nickel
Human urine	Oxidative DNA damage	+	Kim et al. 2004	Nickel
Human plasma	Oxidative DNA damage	+	Wu et al. 2015	Nickel
Human buccal mucosa cells	DNA damage	–	Hafez et al. 2011	Nickel
Human sperm cells	DNA damage	–	Wang et al. 2016a	Nickel

Table 2-5. Genotoxicity of Nickel *In Vivo*

Species (test system)	Endpoint	Results	Reference	Compound
Rat bone marrow and spermatogonial cells	Chromosome aberrations	–	Mathur et al. 1978	Nickel sulfate
Mouse bone marrow cells	Chromosome aberrations (ip)	+	Dhir et al. 1991	Nickel chloride
Mouse bone marrow cells	Chromosome aberrations	+	El-Habit and Abdel Moneim 2014	Nickel chloride
Mouse bone marrow cells	DNA damage	+	El-Habit and Abdel Moneim 2014	Nickel chloride
Mouse leukocytes	DNA damage	+	Danadevi et al. 2004	Nickel chloride
Rat type II lung epithelial cells	DNA damage	+	Oller et al. 2022	Nickel subsulfide
Rat type II lung epithelial cells	DNA damage	–	Oller et al. 2022	Nickel sulfate hexahydrate
Mouse testis and epididymal sperm cells	DNA double-strand breaks	+	Doreswamy et al. 2004	Nickel chloride
Mouse germline sperm cells	DNA double-strand breaks	+	Domshlak et al. 2005	Nickel sulfate
Mouse blood mononuclear cells	DNA fragmentation	+	Jia and Chen 2008	Nickel chloride
Mouse bone marrow cells	Micronucleus test (ip)	–	Morita et al. 1997	Nickel chloride, nickel sulfate, nickel oxide
Rat bone marrow cells	Micronucleus test (oral)	–	Covance Laboratories 2003	Nickel sulfate
Mouse bone marrow cells	Micronucleus test (ip)	–	Deknudt and Léonard 1982	Nickel chloride
Mouse bone marrow cells	Micronucleus test	+	El-Habit and Abdel Moneim 2014	Nickel chloride
Mouse lung, mouse nasal mucosa, rat lung, rat nasal mucosa	Gene mutation (inhalation)	–	Mayer et al. 1998	Nickel subsulfide
Mouse pigment cells	Gene mutations	+	Domshlak et al. 2005	Nickel sulfate
Mouse	Dominant lethal (ip)	–	Deknudt and Léonard 1982	Nickel acetate
Mouse germline sperm cells	Dominant lethal mutations	+	Domshlak et al. 2005	Nickel sulfate

– = negative result; + = positive result; (+) = weakly positive result; ip = intraperitoneal

Table 2-6. Genotoxicity of Nickel *In Vitro*

Species (test system)	Endpoint	Results		Reference	Compound
		With activation	Without activation		
Prokaryotic organisms:					
Bacillus subtilis	DNA damage (rec assay)	NT	–	Kanematsu et al. 1980	Nickel oxide, Nickel trioxide
Escherichia coli	DNA replication rate	NT	+	Chin et al. 1994	Nickel chloride
S. typhimurium	DNA damage	+	–	Keyhani et al. 2006	Nickel
E. coli WP2	Gene mutation frequency	NT	–	Green et al. 1976	Nickel chloride
Salmonella typhimurium	Gene mutation frequency	NT	–	Arlauskas et al. 1985	Nickel chloride, Nickel sulfate
S. typhimurium	Gene mutation frequency	NT	–	Biggart and Costa 1986	Nickel chloride
S. typhimurium TA102	Gene mutation frequency	NT	–	Marzin and Phi 1985	Nickel nitrate
S. typhimurium	Gene mutation frequency	–	–	Wong 1988	Nickel chloride
Cornebacterium sp.	Gene mutation frequency	NT	+	Pikálek and Necásek 1983	Nickel chloride
Eukaryotic organisms:					
Fungi:					
Saccharomyces cerevisiae	Reverse mutation	NT	–	Singh 1984	Nickel sulfate
Mammalian cells:					
Human foreskin cells	Cell transformation	NT	+	Biedermann and Landolph 1987	Nickel subsulfide, Nickel oxide, Nickel sulfate, Nickel acetate
Baby hamster kidney (BHK-21 cells)	Cell transformation	NT	+	Hansen and Stern 1984	Nickel powder, Nickel acetate, Nickel oxide, Nickel subsulfide
Chinese hamster embryo (CHE) cells	Cell transformation	NT	+	Conway and Costa 1989	Nickel chloride, Nickel sulfide
Chinese hamster ovary (CHO) cells	Cell transformation	NT	+	Costa and Heck 1982	Nickel sulfide, Nickel subsulfide, Nickel oxide, metallic Nickel
CHO cells	Cell transformation	NT	+	Costa and Mollenhauer 1980	Nickel sulfide, Nickel subsulfide
CHO cells	Cell transformation	NT	+	Costa et al. 1982	Nickel sulfide

Table 2-6. Genotoxicity of Nickel *In Vitro*

Species (test system)	Endpoint	Results		Reference	Compound
		With activation	Without activation		
Syrian hamster embryo (SHE) cells	Cell transformation	NT	+	Costa and Mollenhauer 1980	Nickel sulfide, Nickel subsulfide
SHE cells	Cell transformation	NT	+	Costa et al. 1982	Nickel sulfide
SHE cells	Cell transformation	NT	+	DiPaolo and Casto 1979	Nickel sulfate, Nickel subsulfide
Mouse embryo cells (C3H/10T1/2)	Cell transformation	NT	+	Saxholm et al. 1981	Nickel subsulfide
Mouse embryo fibroblasts	Cell transformation	NT	+	Miura et al. 1989	Nickel subsulfide, Nickel monosulfide, Nickel oxide
Mouse embryo fibroblasts	Cell transformation	NT	–	Miura et al. 1989	Nickel sulfate, Nickel chloride
Mouse embryo cells	Cell transformation	NT	+	Clemens and Landolph 2003	Nickel arsenide
Human lymphocytes	Chromosome aberration	NT	+	Larramendy et al. 1981	Nickel sulfate
Human bronchial epithelial cells	Chromosome aberration	NT	+	Lechner et al. 1984	Nickel sulfate
Human bronchial epithelial cells	Chromosome aberration	NT	+	Holmes et al. 2013	Nickel subsulfide
Human liver cancer cells	Chromosome aberration	NT	+	Terpilowska and Siwicki 2018	Nickel chloride
Mouse embryo cells	Chromosome aberration	NT	+	Clemens and Landolph 2003	Nickel arsenide
Mouse embryo fibroblasts	Chromosome aberration	NT	+	Terpilowska and Siwicki 2018	Nickel chloride
CHE cells	Chromosome aberration	NT	+	Conway and Costa 1989	Nickel chloride, Ni sulfide
CHO cells	Chromosome aberration	NT	+	Sen and Costa 1986	Nickel chloride, Ni sulfide
CHO cells	Chromosome aberration	NT	+	Sen et al. 1987	Nickel sulfate, Nickel chloride
C3H/10T1/2 cells	Chromosome aberration	NT	+	Sen et al. 1987	Nickel sulfate, Nickel chloride
SHE cells	Chromosome aberration	NT	+	Larramendy et al. 1981	Nickel sulfate
Chinese hamster V79 cells	Chromosome aberration	NT	+	Ohshima 2003	Nickel sulfate
CHO cells	Gene mutation at HGPRT locus	NT	–	Hsie et al. 1979	Nickel chloride

Table 2-6. Genotoxicity of Nickel *In Vitro*

Species (test system)	Endpoint	Results		Reference	Compound
		With activation	Without activation		
Chinese hamster V79 cells	Gene mutation at HGPRT locus	NT	+	Hartwig and Beyersmann 1989	Nickel chloride
Chinese hamster V79 cells	Gene mutation at HGPRT locus	NT	+	Miyaki et al. 1979	Nickel chloride
Chinese hamster V79 cells	Gene mutation at HGPRT locus	NT	–	Åkerlund et al. 2018	Nickel chloride
Chinese hamster V79 cells	Gene mutation at HPRT locus	NT	+	Ohshima 2003	Nickel sulfate
Chinese hamster V79 cells	Gene mutation at HPRT locus	NT	–	Buxton et al. 2020	Nickel metal powder
CHO AS52 cells	Gene mutation at *grp* locus	NT	+	Fletcher et al. 1994	Nickel oxide (black and green); amorphous Nickel sulfide; Nickel subsulfide; Nickel chloride; Nickel sulfate; Nickel acetate
CD2F1 mouse lung and nasal mucosa cells	DNA fragmentation	NT	+	Mayer et al. 1998	Nickel subsulfide
Human diploid fibroblasts	DNA single strand breaks	NT	–	Hamilton-Koch et al. 1986	Nickel chloride
Human gastric mucosal cells	DNA damage (comet analysis)	NT	–[a]	Pool-Zobel et al. 1994	Nickel sulfate
Human HeLa cells	DNA replication	NT	+	Chin et al. 1994	Nickel chloride
Human leukemic cells	DNA damage	NT	–	Cavallo et al. 2003	Nickel sulfate
Human leukemic cells	Inhibition of DNA repair	NT	+	Cavallo et al. 2003	Nickel sulfate
Human leukemic cells	DNA fragmentation	NT	+	Jia and Chen 2008	Nickel chloride
Human lymphoblastoid TK6 cells	DNA damage	NT	+	Guillamet et al. 2008	Nickel chloride
Human B lymphoblastoid cells	DNA damage	NT	+	Lou et al. 2013	Nickel chloride
Human lymphocytes	DNA single strand breaks	NT	+	Chen et al. 2003	Nickel chloride

Table 2-6. Genotoxicity of Nickel *In Vitro*

Species (test system)	Endpoint	Results		Reference	Compound
		With activation	Without activation		
Human lymphocytes	DNA damage	NT	+	Rao et al. 2008	Nickel chloride
Human peripheral lymphocytes	DNA single strand breaks	NT	+	M'Bemba-Meka et al. 2005	Nickel carbonate hydroxide, Nickel subsulfide, Nickel oxide
Human peripheral lymphocytes	DNA single strand breaks	NT	–	M'Bemba-Meka et al. 2005	Nickel sulfate
Human alveolar epithelial cells (A549)	DNA strand breaks	NT	+	Schwerdtle and Hartwig 2006	Nickel chloride, Nickel oxide
Human alveolar epithelial cells	DNA damage	NT	–	Di Pietro et al. 2009	Nickel
Human umbilical cord endothelial cells	DNA damage	NT	+	Beck et al. 2014	Nickel
Human bronchial epithelial cells	DNA fragmentation	NT	+	Castorina and Giunta 2014	Nickel acetate
Human bronchial epithelial cells	DNA strand breaks	NT	+	Di Bucchianico et al. 2018	Nickel chloride
Human bronchial epithelial cells	DNA damage	NT	+	Gliga et al. 2020	Nickel chloride
Human bronchial epithelial cells	DNA damage	NT	–	Åkerlund et al. 2018	Nickel chloride
Human dermal fibroblast cells	DNA strand breaks	NT	+	Belliardo et al. 2018	Nickel chloride
Human colon cancer cells	DNA damage	NT	–	Kim and Seo 2011	Nickel acetate
Human colon cancer cells	DNA damage	NT	–	Kim and Seo 2012	Nickel acetate
Human fetal fibroblast cells	DNA damage	NT	+	Qiao and Ma 2013	Nickel
Human liver cancer cells	DNA damage	NT	+	Terpilowska and Siwicki 2018	Nickel chloride
Human proximal tubule epithelial cells	DNA damage	NT	+	Wang et al. 2012	Nickel acetate
Mouse embryo fibroblast cells	DNA damage	NT	+	Wang et al. 2016b	Nickel
Mouse embryo fibroblast cells	DNA damage	NT	+	Terpilowska and Siwicki 2018	Nickel chloride
Chinese hamster V79 cells	DNA damage	NT	–	Nordin et al. 2018	Nickel

Table 2-6. Genotoxicity of Nickel *In Vitro*

Species (test system)	Endpoint	Results		Reference	Compound
		With activation	Without activation		
CHO cells	DNA protein crosslinks	NT	+	Patierno and Costa 1985	Crystalline Nickel sulfide, Nickel chloride
CHO cells	DNA strand breaks	NT	+	Hamilton-Koch et al. 1986	Nickel chloride
CHO cells	DNA single strand breaks	NT	+	Patierno and Costa 1985	Crystalline Nickel sulfide, Nickel chloride
Rat kidney cells	DNA single strand breaks	NT	+	Chen et al. 2010	Nickel chloride
Human lymphocytes	Metaphase analysis	NT	+	Arrouijal et al. 1992	Nickel subsulfide
Human lymphocytes	Micronucleus formation	NT	+	Arrouijal et al. 1992	Nickel subsulfide
Human bronchial epithelial cells	Micronucleus formation	NT	–	Gliga et al. 2020	Nickel chloride
Human colon cancer cells	Micronucleus formation	NT	–	Kim and Seo 2011	Nickel acetate
Chinese hamster V79 cells	Micronucleus formation	NT	–	Nordin et al. 2018	Nickel
Chinese hamster V79 cells	Micronucleus formation	NT	–	Buxton et al. 2020	Nickel
Human lymphocytes	Sister chromatid exchange	NT	(+)	Andersen 1983	Nickel sulfate
Human peripheral lymphocytes	Sister chromatid exchange	NT	+	Larramendy et al. 1981	Nickel sulfate
Human peripheral lymphocytes	Sister chromatid exchange	NT	+	M'Bemba-Meka et al. 2007	Nickel carbonate hydroxide, Nickel subsulfide, Nickel oxide, Nickel sulfate
Human lymphocytes	Sister chromatid exchange	NT	+	Saxholm et al. 1981	Nickel subsulfide
Human lymphocytes	Sister chromatid exchange	NT	+	Wulf 1980	Nickel sulfate
Human lymphocytes	Sister chromatid exchange	NT	+	Arrouijal et al. 1992	Nickel subsulfide
Chinese hamster V79 cells	Sister chromatid exchange	NT	+	Hartwig and Beyersmann 1989	Nickel chloride

Table 2-6. Genotoxicity of Nickel *In Vitro*

Species (test system)	Endpoint	Results		Reference	Compound
		With activation	Without activation		
Chinese hamster DON cells	Sister chromatid exchange	NT	+	Ohno et al. 1982	Nickel sulfate, Nickel chloride
SHE cells	Sister chromatid exchange	NT	+	Larramendy et al. 1981	Nickel sulfate
Virus-infected mouse sarcoma cells	Induction of revertant foci	NT	+	Biggart et al. 1987	Nickel chloride
Virus-infected mouse sarcoma cells	Induction of revertant foci	NT	+	Biggart and Murphy 1988	Nickel chloride
Mouse lymphoma (L5178Y/TK$^{+/-}$) cells	Forward mutation	NT	+	Amacher and Paillet 1980	Nickel chloride
Mouse lymphoma (L5178Y/TK$^{+/-}$) cells	Forward mutation	NT	+	McGregor et al. 1988	Nickel sulfate

[a]Nickel was genotoxic and cytotoxic at the same concentration (9.5 µmol/mL), so it was not a selective genotoxicant.

– = negative result; + = positive result; (+) = weakly positive result; DNA = deoxyribonucleic acid; NiS = nickel sulfide

2.21 NICKEL NANOPARTICLES

The following section provides a brief overview on toxicity of nickel nanoparticles (NiNPs) and is focused on highlighting findings from experimental animal studies. No epidemiology studies using NiNPs were identified. A case report indicates that a worker developed NiNPs powder sensitization when working in a setting handling 1-2 grams of nano nickel powder without any special respiratory protection or control measures (Journeay and Goldman 2014). In another case report occupational inhalation exposure to NiNPs via spraying resulted in death 13 days after exposure, the cause of death at autopsy was determined to be ARDS (Phillips et al. 2010). The case report by Phillips et al. (2010) also identified high levels of NiNPs in the urine and kidneys which were indicative of acute tubular necrosis. Occupational NiNPs inhalation is associated with increased risk of lung fibrosis, and high incidence of lung and nasal cancer is also reported (Genchi et al. 2020). Several *in vivo* and *in vitro* studies have demonstrated that NiNPs increase the production of reactive oxygen species and reactive nitrogen species

which are both associated in other studies with serious adverse effects such as genotoxicity, inflammation, apoptosis, and fibrosis (Chang et al. 2017; Genchi et al. 2020).

Many studies in animals have reported a wide range of adverse effects in the respiratory system following exposure to NiNPs. Single inhalation exposure to NiO (nickel oxide) NPs at a concentration of 0.00134 mg/m^3 in BALB/C mice for 4 hours resulted in nidal perivascular and peribronchial lymphoid infiltration in the lungs of the exposed mice (Zaitseva et al. 2019). This study also observed changes in alveolar patterns in mice exposed to NiNPS. Wistar rats were exposed to NiO NPs via intratracheal instillation twice a week for 6 weeks at 0.24 mg/kg-bodyweight, which induced abnormal changes in hepatic enzymes (Yu et al. 2018). Single intratracheal instillation of NiO NPs in male Sprague-Dawley rats to a concentration of 800 µg (3.3 mg/kg) induced pulmonary inflammation with elevated neutrophil count (Cao et al. 2016). Single intratracheal instillation of NiNPs at 5.6 mg/kg in Sprague-Dawley rats caused hepatotoxicity (Magaye et al. 2016). Single intratracheal instillation of NiO NPs in Wistar rats at the concentration of 5 mg/ml resulted in lung injury and oxidative stress over a period of 72 hours after the exposure (Horie et al. 2012). C57BL/6N mice inhaled 0.5 mg/ml NiO NPS mist by nasal exposure 4 times/day (10 min/day) for 8 days with a 1 week break after the first 4 days; this treatment induced pulmonary inflammation and an immune response by increasing the expression of IgE (Horie et al. 2016). Whole body inhalation exposure to NiNPs at a concentration of 500 $\mu g/m^3$ for 5 hours in C57BL/6 mice resulted in significantly increased circulating endothelial progenitor cells, indicating endothelial damage caused by NiNPs (Liberda et al. 2014). Whole body inhalation exposure to nickel sulfate ($NiSO_4$) NPs at a concentration of 558 $\mu g/m^3$ in mice for 4 hours resulted in pulmonary inflammation (Kang et al. 2011a). Whole body inhalation exposure to nickel hydroxide (NH) NPs at a concentration of 79 $\mu g/m^3$ for 5 hours/day, 5 days/week, for 1 week in hyperlipidemic, apoprotein E-deficient ($ApoE^{-/-}$) mice resulted in increased oxidative stress, cardiopulmonary inflammation, DNA damage in aorta, significant signs of inflammation in bronchoalveolar lavage fluid, and changes in lung histopathology (Kang et al. 2011b). A five-month exposure in the same study exacerbated the health effects observed in the 1 week exposure (Kang et al. 2011b). Whole body inhalation of NH NPs in C57BL/6 mice for 5 hours/day for one day induced acute endothelial disruption and caused vasoconstriction at 150 $\mu g/m^3$; this effect occurred after 3- and 5- day exposures as well (Cuevas et al. 2010). Male Fischer-344 rats received NiO NPs as 4 doses of 2 mg/kg/bw as intratracheal instillations which caused pulmonary injury and inflammation, and NiO particles were detected in the lung and lung associated lymph nodes (Senoh et al. 2017). Male Wistar rats were subjected to two aerosol inhalation exposures of NiO NPs for 6 hours/day, 5 days/week for 4 weeks at 0.20 mg/m^3 which resulted in macrophage accumulation in the alveoli with infiltration of inflammatory cells (Kadoya et al. 2016). Albino rats were exposed to NiO NPs at 0.23 mg/m^3 for 4 hours/day, 5 times a week for up to 10 months and resulted in altered pulmonary cytology and biochemical characteristics of

the bronchoalveolar lavage fluid (Sutunkova et al. 2019). Sutunkova et al. (2019) also observed damage to the liver and kidneys along with genotoxic effects assessed by the increased degree of DNA fragmentation. In male Wistar rats exposed to NiO NPs via intratracheal instillation, twice a week for 6 weeks at 0.24 mg/kg bw, increased indicators of nitrative stress (NO, TNOS, and iNOS), inflammatory cytokines (TNF-a, IL-2, and IL-10), and cytokine induced neutrophil chemoattractants (CINC-1, CINC-2ab, and CINC-3) were observed in lung tissue (Chang et al. 2017). NiO NPs when intratracheally instilled into female Wistar rats at 200 cm^2/rat produced an acute neutrophilic inflammation (Lee et al. 2016b). Male Wistar rats were exposed to 0.2 mg NiO NPs via intratracheal instillation once which caused a persistent inflammatory effect, and a transient increase in cytokine expression and persistent pulmonary inflammation (Morimoto et al. 2011; Morimoto et al. 2016; Morimoto et al. 2010). A 4-week intratracheal instillation of 0.1-3 mg NiO NPs in male Wistar rats caused pulmonary inflammation (Mizuguchi et al. 2013; Ogami et al. 2009). A dose-dependent increase in acute lung inflammation and injury was seen in C57BL/6 mice after exposure to 50 µg NiNPs via intratracheal instillation (Mo et al. 2019).

Oral exposure to NiO NPs in animals primarily targets both male and female reproductive organs and the immune system. Oral exposure to 100 mg/kg-bodyweight nickel oxide NPs in water to pregnant albino rats for 12 to 14 days of gestation significantly increased luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone hormones (Alsoltane and Altaee 2020b). Kong et al. (2019) orally dosed Sprague-Dawley rats with NiNPs via food for 10 weeks and examined reproductive toxicity in one generation. At 15 mg/kg-bw, NiNPs induced oxidative stress and caused morphological changes in the testis (Kong et al. 2019). At the same dose, female Sprague-Dawley rats showed slight swelling, cavitation, and crest disorders of mitochondria in primary follicles along with increased oxidative stress and cell apoptosis (Kong et al. 2016). Kong et al. (2014) observed transgenerational effects in F0 generation on reproductive toxicity in male and female rats dosed with 5 to 15 mg/kg-bw. Male rats showed morphological changes in the testis while female rats showed changes in hormone levels. Developmental toxicity was observed in the pups with a significant decrease in survival rates at birth and during feeding (Kong et al. 2014). Oral exposure to 100 mg/kg bodyweight NiO NPs in water to pregnant albino rats for 12 to 14 days of gestation significantly decreased IgA, IgG, and IgM (Alsoltane and Altaee 2020b). A single oral NiO NP dose of 500 mg/kg via intubation in adult Wistar rats resulted in increased white blood cell count (Dumala et al. 2018).

Effects in several other systems have been reported in various animal studies. In male Wistar rats orally exposed to NiO NPs at 2 mg/kg-bw/day, significant increases in chromosomal aberrations, micronuclei formation, and DNA damage were induced after 7-day and 14-day exposures (Saquib et al. 2017). Oral

exposure to 100 mg/kg-bw NiO NPs in water to pregnant albino rats for 12 to 14 days resulted in decreased maternal relative body weight. Exposed rats on gestation day 12 showed an increase in relative organ weight (lung, uterus, kidney) and decreases in heart, liver, eye, spleen, and brain weights. Similarly, decreases in relative weight of the heart, liver, eye, brain, and kidney and increases in lung, spleen, and uterus weight were observed in treated rats on gestation day 14 (Alsoltane and Altaee 2020a). At lower doses, Wistar rats exposed to NiO NPs via food for 14 days at 0.5 and 1 mg/kg-bw showed increases in relative weight of the brain, kidney, and liver, and increases in erythrocytes and hemoglobin levels (Ali 2019). Changes in kidney and liver enzymes were also noted. Hematological effects were observed in Wistar rats after 28-days of repeated oral exposure to NiO NPs, including decreased hemoglobin and hematocrit levels in male and female rats exposed to ≥50 mg/kg-bw (Dumala et al. 2019b).

Parenteral exposure to NiNPs targets the hematological system, heart, kidneys, and liver. Exposure to 5 mg/kg-bw NiNPs in male ICR mice by intraperitoneal injection damages the reproductive system by affecting spermatogenesis and testicular structure (Hu et al. 2020). Adult male Wistar rats exposed to 25 mg/kg-bw NiNPs and nickel chloride intraperitoneally daily for 1 week developed a significant increase in blood urea, creatinine, and white blood cell count (Seyedalipour et al. 2017). Wistar rats dosed with NiO NPs via intraperitoneal injection at 2.5 mg/kg for 3 times a week up to 18 injections, developed decreased hematocrit levels and lymphocytes and increased monocytes and reticulocytes along with morphological changes observed in the brain, kidney, liver, and spleen (Minigalieva et al. 2015). Intraperitoneal injections of 20-50 NiO NPs mg/ml for 14 days in albino mice induced oxidative stress that affected cardiac, hepatic, and renal systems. The effects were dose and sex dependent as they were more pronounced at higher doses and specifically in male mice (Hussain et al. 2020).

The genotoxic effects of NiNPs have been tested in *in vivo* and *in vitro* studies. DNA damage, increased polychromatic erythrocytes in the micronucleus test, and chromosomal aberrations were seen in female Wistar rats orally exposed to 2,000 mg/kg/bw of NiO NPs once (Dumala et al. 2017). Peripheral blood lymphocytes isolated from humans showed dose-dependent cytotoxic and genotoxic effects when exposed to NiO NPs for 24 hours (Dumala et al. 2019a). No cytotoxicity was observed in human bronchial epithelial cells exposed to doses up to 50 µg/ml of NiNPs and NiO NPs for 24 hours (Åkerlund et al. 2018, 2019). In Åkerlund et al. (2018), NiNPs and NiO NPs induced DNA strand breaks at doses of 5 to 25 µg/ml. NiO NPs appear more toxic; DNA damage began at 5 µg/ml compared to 10 µg/ml from NiNP exposure (Åkerlund et al. 2018). However, double strand breaks were not significantly increased. Significant differences in the frequencies of micronuclei, which is indicative of genotoxic potential, occurred in both Chinese hamster cell lines and *D.melanogaster* exposed to NiO NPs concentrations of 250 and 500 µg/mL for 4- and 24-hour treatment periods (De Carli et al. 2018). These effects were also

seen at 125 µg/mL NiO NPs only in the 4-hour exposure period (De Carli et al. 2018). A comet assay of V79 cells revealed that 62, 125, 250 and 500 µg/mL NiO NPs induced a significant increase in DNA damage (De Carli et al. 2018). The results from De Carli et al. (2018) indicate that NiO NPs are genotoxic and mutagenic *in vitro* and *in vivo.* Exposure to NiNPs induced genotoxic effects and increased oxidized stress in immortalized human bronchial epithelial (BEAS-2B) cells at doses as low as 1 µg/ml after 48 hours (Di Bucchianico et al. 2018). Low dose NiNPs and NiO NPs exposure at 0.5 µg/mL on BEAS-2B cells for 6 weeks resulted in DNA strand breaks on comet assay (Gliga et al. 2020). Cytotoxicity and DNA strand breaks in a Chinese hamster lung fibroblast cell line occurred after a 48-hour exposure at 0.15 µg/cm^2 and oxidative stress in a human type II alveolar epithelial cell line exposed to 10 µg/ml NiNPs (Latvala et al. 2017; Latvala et al. 2016). Lung tissues exposed to 5-25 µg/cm^2 NiNPs showed dose-dependent cytotoxicity (Magaye et al. 2016). Dose-dependent cyto- and geno- toxicity of NiNPs and NiO NPs was observed in human lung epithelial cells, liver HepG2 cells, human skin epidermal cells, intestinal epithelial cells, and breast MFC-7 cancer cells mediated through oxidative stress (Abudayyak et al. 2020; Ahamed 2011; Ahamed et al. 2015; Ahmad et al. 2015; Alarifi et al. 2014; Capasso et al. 2014; Duan et al. 2015; Saquib et al. 2018). Dose-dependent genotoxicity to nickel nanomaterials was observed in *D. melanogaster* after 24 hours of exposure (Alaraby et al. 2018).

Research on the absorption of NiNPs is limited, but existing data shows that smaller nickel particles are absorbed more readily than larger ones. This suggests that absorption rates may be higher for NiNPs than for other nickel compounds due to their small size. Solubility of NiNPs may be related to shape. In a study of intratracheal exposure in rats, spherical NiO NPs dissolved less readily in artificial lysosomal fluid and had lower pulmonary clearance rates than wire-shaped NiO NPs, suggesting that wire-shaped NiNPs may be more readily absorbed by the lungs. The smallest NiO NPs also had the highest absorption and distribution rates (Shinohara et al. 2017). NiNP shape may also affect distribution rate. In a study of differently shaped NiNPs administered intratracheally to rats, distribution from the lungs to lymph nodes was time- and dose-dependent for spherical and irregular NiO particles, but not for wire-shaped ones (Shinohara et al. 2017). Dumala et al. (2018) also observed that a single oral dose of 125 mg/kg-bw NiO NPs in rats accumulated in the blood, liver, and kidney and the 250 mg/kg-bw dose in the brain. Rat neuronal cells exposed to NiO NPs 0-500 µg/ml for 24 hours resulted in a dose-dependent uptake of the nanoparticles and DNA damage, decreased cell viability, and oxidative stress (Abudayyak et al. 2017b). In another study, similar doses of NiO NPs in kidney epithelial cells resulted in DNA damage and apoptosis (Abudayyak et al. 2017a). NiNPs accumulated in the liver and spleen of Wistar rats dosed with 2.5 mg/kg NiO NPs via intraperitoneal injection 3 times a week up to 18 injections (Minigalieva et al. 2015). In a study by Shinohara et al. (2017) pulmonary clearance rate constants were estimated using a one-compartment model in rats which demonstrated that the shape of NiNPs influenced the clearance.

Spherical and irregular shaped NiO NPs showed time- and dose-dependent increases in translocation from lungs to the thoracic lymph nodes, but wire-like NiO NPs did not (Shinohara et al. 2017).

There is little data about the metabolism of NiNPs, but research suggests NiNPs have the same target organs as larger nickel compounds and exert toxicity in a similar manner (binding to ligands in serum).

NiO NPs appear to be excreted via urine and feces and appear to be dose- and time-dependent (Dumala et al. 2018). In this study, the excretion of nickel in urine was significant at all doses of NiO NPs at all sampling times in a dose- and time-dependent manner. In feces, the maximum amount of NiO NPs was cleared significantly and clearance was rapid from 18 to 24 hours (Dumala et al. 2017). Wistar rats were dosed with NiO NPs via intraperitoneal injection at a dose of 2.5 mg/kg 3 times a week up to 18 injections and NiO NPs underwent renal excretion (Minigalieva et al. 2015). Whole body inhalation exposure to NiO NPs for 6 hours/day for 4 weeks resulted in accumulation of NiO NPs in the lungs; retained particles in rat lungs after inhalation exponentially decreased with a calculated biological half time of 62 days (Oyabu et al. 2007). In a study of differently shaped NiNPs administered intratracheally to rats, wire-shaped NiO NP were excreted in urine much more quickly (35% 24-hours after administration) than spherical and irregular particles (0.33-3.6% 24-hours after administration) (Shinohara et al. 2017).