CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of acrolein. 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. Mechanisms of action are discussed along with the health effects data for respiratory, immunological and cancer outcomes. An overview of general mechanisms that contribute to multiple health effects is provided in Section 2.21 and toxicokinetic mechanistic data are discussed in Section 3.1.

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

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

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to acrolein, 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 acrolein was also conducted; the results of this review are presented in Appendix C.

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

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. Effects have been classified into “less serious LOAELs” or “serious LOAELs (SLOAELs).” “Serious” effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g.,

acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health. Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of acrolein are indicated in Table 2-1 and Figure 2-2.

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.

The health effects of acrolein have been evaluated in 20 human studies and 102 animal studies. As illustrated in Figure 2-1, most of the health effects data come from inhalation studies in animals. The ocular effects observed in acrolein inhalation studies are likely attributable to direct contact with acrolein vapors. Therefore, ocular effects from inhalation studies are counted as dermal exposure in Figure 2-1 and are listed in the dermal LSE table. For animal data, inhalation and oral studies are available for most health effects and exposure duration categories. The dermal animal database is limited to ocular effects, mostly after exposure to acrolein vapor. The most examined endpoints in animal studies were respiratory, death, body weight, hepatic and cardiovascular. The available human studies were predominantly focused on evaluation of respiratory and ocular effects.

A systematic review was conducted on potential toxicity targets of acrolein exposure, which included respiratory and immunological effects for inhalation exposure and gastrointestinal effects following oral exposure (see Appendix C for details).

- **Respiratory Effects.** Respiratory effects are a presumed health effect associated with acrolein exposure via inhalation based on moderate evidence in humans and a high level of evidence in animals. Rapid onset of nose and throat irritation and a reduction in breathing rate (believed to be a protective measure triggered by nose irritation) was reported by volunteers acutely exposed to

low levels of acrolein. Epidemiology studies have also reported associations between acrolein exposure and reporting of respiratory irritation symptoms, prevalence of asthma and decrements in pulmonary function. Numerous animal studies have reported nasal and pulmonary lesions, altered respiratory function and increased lung weight following acute-, intermediate-, and chronic-duration inhalation studies in rodents. While the entire respiratory tract may be affected by acrolein inhalation, the nasal epithelium appears to be more sensitive at lower exposures (<1 ppm), which is consistent with human perception of nasal irritation. The deeper respiratory regions (bronchiolar and alveolar regions) appear to be sensitive to higher exposure levels, with severe effects being observed from exposures of ≥100 ppm.

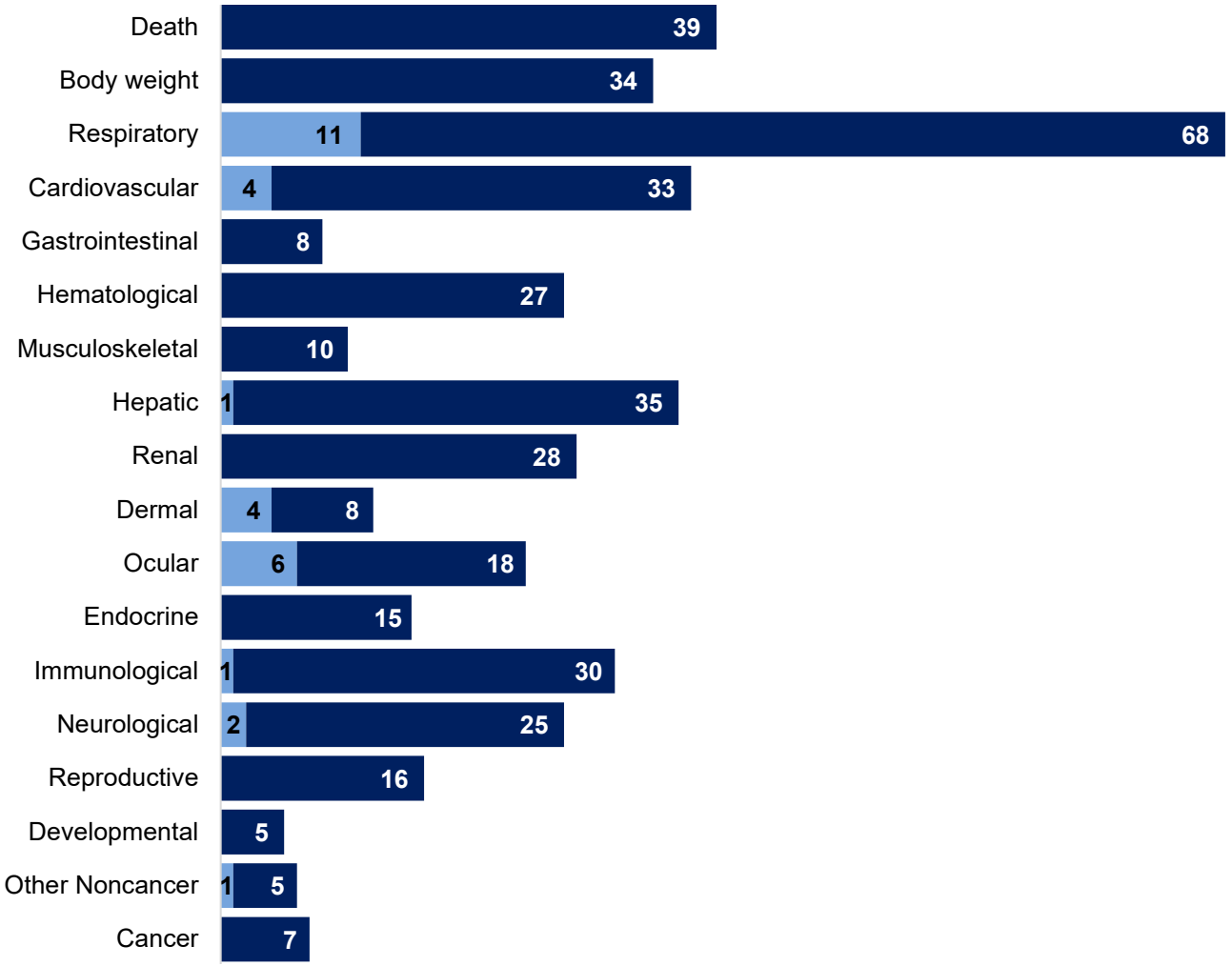
- **Immunological Effects.** Immunological effects following inhalation exposure are a suspected health effect based on a moderate level of evidence in animal studies; there is inadequate evidence in humans to make a conclusion. Although histological changes were not observed in immune organs (spleen, thymus) following inhalation, or in some cases oral exposure, acrolein exposure appears to alter immune function. Following inhalation of acrolein, several studies have reported decreased bactericidal activity, decreased numbers of alveolar macrophages, increased mortality from pulmonary bacterial infection, or suppression of the pulmonary immune response to ovalbumin challenge.

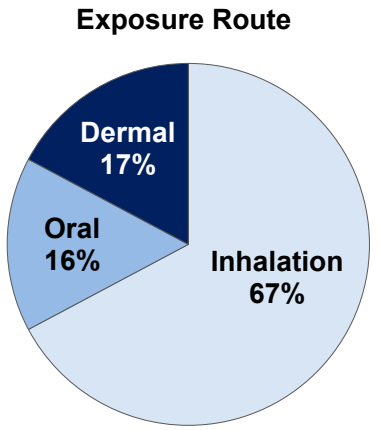
- **Gastrointestinal Effects.** Gastrointestinal effects following oral exposure are a suspected health effect based on a moderate level of evidence in animal studies; there is inadequate evidence in humans to make a conclusion. In animals, stomach lesions including ulcers, hemorrhage, hyperplasia of the forestomach, and/or erosion of the glandular mucosa were seen after intermediate-duration exposure. No histological changes were seen in rodents or dogs after chronic-duration oral exposure (2–4.5 mg/kg/day) suggesting possible adaptation to irritating effects may have occurred.

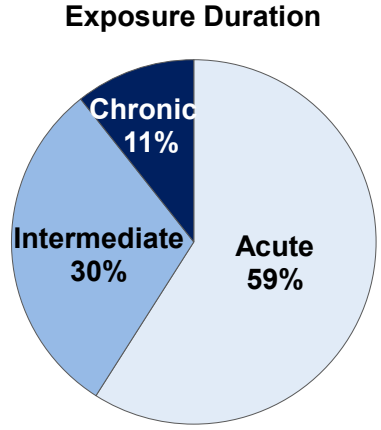
Figure 2-1. Overview of the Number of Studies Examining Acrolein Health Effects*

Most studies examined the potential respiratory effects of acrolein

Fewer studies evaluated health effects in humans than animals (counts represent studies examining endpoint)







*Includes studies discussed in Chapter 2. A total of 122 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

Table 2-1. Levels of Significant Exposure to Acrolein – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Dwivedi et al. 2015									
1	Human 9 M, 9 F	2 hours	0, 0.05, 0.11	CS, OF, HP	Resp	0.11			
					Immuno	0.11			
Weber-Tschopp et al. 1977									
2	Human 21 M, 25 F	1 hour	0, 0.3	CS	Resp		0.3[b]		Nose and throat irritation (subjective symptoms); decreased respiratory rate
Arumugam et al. 1999a									
3	Rat (Wistar) 5 M	4 hours	0, 1, 2	HP	Resp		2		Desquamized cells and isolated peribronchial mononuclear cells in the bronchioles, hyperemia, emphysema
Babiuk et al. 1985									
4	Rat (Fischer-344) 4 M	10 minutes	0.5–10.0	OF	Resp		6		RD_{50}
Ballantyne et al. 1989									
5	Rat (Sprague-Dawley) 5 M, 5 F	1 hour	14, 22, 24, 31, 81	LE	Death			24 M 22F	2/5 males and 1/5 females died
					Bd wt	24			
					Resp		14	24 M 22F	LOAEL: Decreased breathing rate and conversion to audible and mouth breathing SLOAEL: congestion and intra-alveolar hemorrhage; fibrin deposition in the smaller airways; necrosis and exfoliation of bronchiolar epithelium in animals that died

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Ballantyne et al. 1989									
6	Rat (Sprague-Dawley) 5 M, 5 F	4 hours	4.8, 7, 9.1, 12.1	LE	Death			7.0 M 9.1 F	3/5 males and 4/5 females died
					Bd wt	7.0		9.1	Body weight loss 7 days after exposure (27 g in males, 18 g in females relative to pre-exposure weight)
					Resp		4.8	7.0 M 9.1 F	LOAEL: Decreased breathing rate and conversion to audible and mouth breathing SLOAEL: congestion and intra-alveolar hemorrhage; fibrin deposition in the smaller airways; necrosis and exfoliation of bronchiolar epithelium in decedents
Bergers et al. 1996									
7	Rat (Wistar) 4 M	20 minutes (N)	6.7, 13.4, 26.9, 53.8	CS, OF	Resp		4.6		RD_{50}
Cassee et al. 1996a									
8	Rat (Wistar) 5–6 M	6 hours	0, 0.67, 1.4	CS, HP	Resp	1.4			
Cassee et al. 1996a									
9	Rat (Wistar) 5–6 M	3 days 6 hours/day	0, 0.25, 0.67, 1.4	CS, HP	Resp		0.25		Disarrangement and thickening of the nasal epithelium, and basal cell hyperplasia
Cassee et al. 1996b									
10	Rat (Wistar) 4 M	30 minutes	1.73, 11.18, 31.9	CS, OF	Resp		9.2		RD_{50}

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Dorman et al. 2008									
11	Rat (F344) 12 M	4–14 days	0, 0.018, 0.052, 0.200, 0.586, 1.733	LE, CS, BW, HP	BW	0.586	1.733		≥10% decreased body weight after 4–6 days
					Resp	0.2	0.586		Mild nasal epithelial hyperplasia in the dorsal meatus and lateral wall, and respiratory epithelial squamous metaplasia in the septum after 4 days
Hazari et al. 2008									
12	Rat (Sprague-Dawley) 6 M	3 hours (WB)	0, 3	OF	Resp		3		Upper respiratory tract (increased pause between the end inspiration and start of expiration) and pulmonary or lower airway (increased pause between end of expiration and start of inspiration) irritation, decreased breathing frequency
					Cardio		3		Decreased heart rate
Kunkler et al. 2018									
13	Rat (Sprague-Dawley) 5–10 M	4 days 4 hours/day (WB)	0, 0.3	CS, NX	Neuro		0.3		Altered pain thresholds and behaviors (increased time spent in corners)
Morris 1996									
14	Rat (Fischer-344) 16–25 M	40 minutes	0, 0.9, 4.5, 9.1	BC	Resp	4.5	9.1		Increased albumin in nasal lavage fluid

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Murphy 1965									
15	Rat (Holtzman) 22 M	4 hours (WB)	0, 8	BI, OW, OF	Resp		8		Increased relative lung weight, pulmonary edema, inflammation
					Hepatic	8			
Murphy et al. 1964									
16	Rat (Sprague-Dawley) 20 M	4 hours	0, 12	CS, BC, BI	Resp			12	Severe respiratory tract irritation, gasping, dyspnea, decreased alkaline phosphatase activity in the lungs
Perez et al. 2013									
17	Rat (SH) 5–6 M	3 hours (WB)	0, 3	OF	Resp		3		Increased breathing frequency and minute volume
					Cardio		3		Increased heart rate and blood pressure
Perez et al. 2013									
18	Rat (WKY) 5–6 M	3 hours (WB)	0, 3	OF	Resp	3			
					Cardio		3		Increased blood pressure
Perez et al. 2015									
19	Rat (SH) 5–20 M	3 hours (WB)	0, 2.9	BI, OF	Resp		2.9		Decreased breathing frequency, increased expiratory time
					Cardio		2.9		Decreased arterial blood oxygen, increased arterial blood carbon dioxide and blood pressure
Perez et al. 2015									
20	Rat (WKY) 5–20 M	3 hours (WB)	0, 2.9	BI, OF	Cardio	2.9			

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Skog 1950									
21	Rat (NS) 8 NS	30 minutes	44–305	CS, GN, HP	Death			130	LC_{50}
					Resp			44	Respiratory difficulties, lung edema, hyperemia, and hemorrhages, degenerative changes in the bronchial epithelium
					Cardio		44		Heart hyperemia
					Hepatic		44		Liver hyperemia
					Renal		44		Kidney hyperemia
					Immuno	44			
					Neuro	44			
Snow et al. 2017									
22	Rat (Wistar) 6 M	1–2 days 4 hours/day (N)	0, 1.97, 4.00	BI, OF	Resp	1.97	4		Nasal and pulmonary inflammation, increased inspiratory and expiratory time, labored breathing
					Hemato	4			
					Hepatic	1.97	4		Increased cholesterol
					Endocr	1.97	4		Increased plasma corticosterone
					Other noncancer	1.97	4		Altered glucose tolerance
Snow et al. 2017									
23	Rat (GK) 6 M	1–2 days 4 hours/day (N)	0, 1.97, 4.00	BI, OF	Resp	1.97	4		Nasal and pulmonary inflammation, increased inspiratory and expiratory time, labored breathing
					Hemato	4			
					Hepatic	1.97	4		Increased cholesterol
					Endocr	4			

Table 2-1. Levels of Significant Exposure to Acrolein – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Other noncancer	1.97	4		Altered glucose tolerance
Aranyi et al. 1986									
24	Mouse (CD-1) 18 F	5 days 3 hours/day	0, 0.1	IX	Immuno		0.1		Decreased bactericidal activity in the lungs
Aranyi et al. 1986									
25	Mouse (CD-1) 24 F	3 hours	0, 0.09	IX	Immuno	0.09			
Astry and Jakab 1983									
26	Mouse (Swiss-Webster) 6 F	8 hours	0, 3.0, 6.0	IX	Immuno		3		Decreased bactericidal activity in the lungs
Bein et al. 2021									
27	Mouse (B6C3F1) 20–32 M, 32–148 F	30 minutes (WB)	0, 50, 75	LE, GN, HP	Death			75 F	Increased mortality (79%)
								50 M	Increased mortality (60%)
					Resp	50 F	75 F		Alveolar wall thickening, proteinaceous deposit and leukocyte infiltrates in the lung
							50 M		Alveolar wall thickening, proteinaceous deposit and leukocyte infiltrates in the lung
Buckley et al. 1984									
28	Mouse (Swiss-Webster) 8–24 M	5 days 6 hours/day	0, 1.7	HP	Resp		1.7		Ulceration, necrosis, and squamous metaplasia of the respiratory and olfactory epithelium in the nasal passages

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Conklin et al. 2017a									
29	Mouse (C57BL/6) 4–23 M, 4–24 F	10–30 minutes (WB)	0, 100, 175, 210, 250, 275	LE, HE, BC, HP, OF	Death			225 M	LC_{50} (30 minutes)
					Resp			250	Labored breathing, gasping, nasal and tracheal lesions (epithelial sloughing, mucus accumulation, inflammatory cell infiltration), increased relative lung weights (males only)
					Cardio	250 F	250 M		Decreased blood oxygen saturation and cardiac output
					Hemato	250 F	250 M		Increased lymphocytes and decreased neutrophils
					Hepatic		250		Increased serum triglycerides
					Other noncancer		250		Decreased body temperature
Danyal et al. 2016									
30	Mouse (C57BL/6J) 6–16 NS	4 hours (WB)	0, 5	IX	Immuno		5		Suppressed inflammation response (reduced airway cytokine response to allergen challenge)
Kane and Alarie 1977									
31	Mouse (Swiss-Webster) 4 M	10 minutes	0, 0.5, 1.7	OF	Resp		1.7		RD_{50}
Kasahara et al. 2008									
32	Mouse (C57BL/6J) 3–6 M	3 days 6 hours/day (WB)	0, 5	HP, IX	Resp	5			
					Immuno	5			

Table 2-1. Levels of Significant Exposure to Acrolein – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Kim et al. 2019									
33	Mouse (BALB/c) 8 F	10 minutes (NS)	0, 5	IX	Immuno		5		Increased airway inflammatory cells after exposure and after OVA challenge
Kim et al. 2020									
34	Mouse (C57BL/6) 3–6 M	12 hours (WB)	0, 10	HP	Resp		10		Air space enlargement in lungs
Kurhanewicz et al. 2017									
35	Mouse (C57BL/6) 8–12 F	3 hours (WB)	0, 3	OF	Cardio		3		Increased heart rate variability, number of arrhythmias, and left ventricle pressure
Kurhanewicz et al. 2018									
36	Mouse (C57BL/6) 6–8 F	3 hours (WB)	0, 3	OF	Resp		3		Increased expiratory time, tidal volume, and enhanced pause, decreased breathing frequency
					Cardio		3		Increased heart rate variability and the number of arrhythmias
Leikauf et al. 2011									
37	Mouse (129X1/ SvJ) 6–16 F	6–17 hours (NS)	0, 10	HP	Resp		10		Perivascular air space enlargement and leukocyte infiltration in the lungs
Leikauf et al. 2011									
38	Mouse (SM/J) 6–16 F	6–17 hours (NS)	0, 10	HP	Resp		10		Perivascular air space enlargement and leukocyte infiltration in the lungs

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Morris et al. 2003									
39	Mouse (C57BL/6N) 4–6 B	10 minutes	0, 1.3	CS	Resp		1.3		Decreased respiratory rate and increased expiratory pause and specific airway resistance
Morris et al. 2003									
40	Mouse (C57BL/6N) 3–6 B	10 minutes	0.3, 1.6, 3.9	CS	Resp		1.59		RD_{50}
Nielsen et al. 1984									
41	Mouse (CF-1) 35 M	30 minutes	0, 0.85, 1.27, 3.0, 7.25	OF	Resp		2.9		RD_{50}
O'Brien et al. 2016									
42	Mouse (C57BL/6) 5–14 M	2 weeks 4 days/week (WB)	0, 5	HP, IX	Resp Immuno	5 5			
Sithu et al. 2010									
43	Mouse (C57BL/6J) 6–8 M	6 hours (WB)	0, 4.9	HE, BC	Hemato Musc/skel Hepatic	 4.9 4.9	4.9		Platelet aggregation
Sithu et al. 2010									
44	Mouse (C57BL/6J) 6–8 M	4 days 6 hours/day (WB)	0, 1.1	HE, BC	Hemato Musc/skel Hepatic	 1.1 1.1	1.1		Platelet aggregation
Spiess et al. 2013									
45	Mouse (C57BL/6) 3–10 M	4 days 6 hours/day (WB)	0, 5	IX	Immuno		5		Suppressed allergic airway inflammatory response following OVA challenge

Table 2-1. Levels of Significant Exposure to Acrolein – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Steinhagen and Barrow 1984									
46	Mouse (Swiss-Webster) 12–16 M	10 minutes	0.04, 0.22, 1.49, 4.92	CS, OF	Resp		1.03		RD_{50}
Steinhagen and Barrow 1984									
47	Mouse (B6C3F1) 12–16 M	10 minutes	0.08, 0.35, 2.68, 8.11	CS, OF	Resp		1.41		RD_{50}
Davis et al. 1967									
48	Guinea pig (NS) 6 NS	60 minutes	0, 17	OF	Resp		17		Decreased respiration rate
Murphy et al. 1963									
49	Guinea pig (NS) 10–14 M	2 hours	0, 0.6	OF	Resp		0.6		Increased respiratory flow resistance and tidal volume, decreased respiration rate
INTERMEDIATE EXPOSURE									
Lyon et al. 1970									
50	Monkey (Squirrel) 7–9 M	6 weeks 5 days/week 8 hours/day	0, 0.7, 3.7	CS, BW, BC, BI, HP, OF	Death			3.7	Increased mortality (2/7)
					Bd wt	3.7			
					Resp		0.7		Chronic inflammation in the lungs
					Hemato	3.7			
					Hepatic	3.7			
Lyon et al. 1970									
51	Monkey (Squirrel) 8–17 M	90 days 24 hours/day	0, 0.22 (0.21 and 0.23 combined), 1.0, 1.8	CS, BW, BC, BI, HP, OF	Bd wt	1.8			
					Resp		1.8		Tracheal squamous metaplasia and basal cell hyperplasia
					Hemato	1.8			

Table 2-1. Levels of Significant Exposure to Acrolein – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Bouley et al. 1975									
52	Rat (OFA) 10–25 M	15-180 days 7 days/week 24 hours/day	0, 0.55	CS, BW, FI, BI, OW, DX	Bd wt		0.55		Decreased body weight (11%)
					Resp		0.55		Nasal irritation (sneezing)
					Immuno		0.55		Decreased number of alveolar macrophages and increased mortality from bacterial infection (after 18 days of exposure)
Bouley et al. 1975									
53	Rat (OFA) 3 M, 21 F	26 days 7 days/week 24 hours/day	0, 0.55	BW, RX, DX	Repro	0.55			
					Develop	0.55			
Costa et al. 1986; Kutzman et al. 1985; NTP 1981									
54	Rat (Fischer-344) 24 M, 24 F	62 days 5 days/week 6 hours/day	0, 0.4, 1.4, 4.0	CS, BW, BI, HP	Death			4 M	Increased mortality (56%)
					Bd wt	1.4			
							4 F		Decreased body weight (12%)
								4 M	Decreased body weight (27%)
					Resp	0.4	1.4	4	LOAEL: bronchiolar epithelial necrosis SLOAEL: Decreased pulmonary function, increased relative lung weight, pulmonary lesions (bronchiolar epithelial necrosis, bronchiolar edema fluid), acute rhinitis, and tracheal edema
					Cardio	4			
					Hemato	4			
					Renal	4			

Table 2-1. Levels of Significant Exposure to Acrolein – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Neuro	4			
					Repro	4			
Dorman et al. 2008									
55	Rat (Fischer-344) 12 M	13 weeks 5 days/week 6 hours/day (WB)	0, 0.018, 0.052, 0.200, 0.586, 1.733	LE, CS, BW, HP	Bd wt	0.59		1.73	Decreased body weight (20%) at the end of exposure
					Resp	0.2	0.59		Nasal respiratory epithelial hyperplasia and squamous metaplasia, laryngeal respiratory squamous metaplasia
Feron et al. 1978									
56	Rat (Wistar) 6 M, 6 F	13 weeks 5 days/week 6 hours/day	0, 0.4, 1.4, 4.9	BW, FI, BC, BI, UR, OW, HP	Death			4.9	Increased mortality (6/12)
					Bd wt	0.4	1.4 F		Decreased body weight (13%)
							1.4 M		Decreased body weight (15%)
					Resp	0.4	1.4	4.9	SLOAEL: Lung lesions (patchy consolidation, collapsed dark areas, hemorrhages, bronchitis, hyperplasia, metaplasia), nasal lesions (necrotizing rhinitis, neutrophilic infiltration),tracheal lesions (severe damage, epithelial metaplasia). Alveolar edema in deceased animals. LOAEL: Nasal squamous metaplasia, and neutrophilic infiltration
					Cardio	4.9			
					Hemato	4.9			
					Hepatic	4.9			
					Renal	4.9			

Table 2-1. Levels of Significant Exposure to Acrolein – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Endocr	4.9			
					Immuno	4.9			
					Neuro	4.9			
					Repro	4.9			
Kutzman et al. 1984									
57	Rat Dahl (hyper-tension-resistant) 10 F	62 days 5 days/week 6 hours/day	0, 0.39, 1.4, 3.96	CS, BW, BI, HP, OW	Death			3.96	Increased mortality (40%)
					Bd wt	1.40		3.96	Decreased body weight (23%)
					Resp	0.39	1.40	3.96	LOAEL: Increased relative lung weights; bronchiolar hyperplasia, peripheral lymphoid aggregation, and macrophage clusters SLOAEL: Pulmonary edema and interstitial pneumonitis
					Cardio	1.40	3.96		Increased relative heart weight
					Hepatic	1.40	3.96		Increased relative liver weight; increased serum ALT, ALP, and AST levels
					Renal	3.96			
					Neuro	3.96			

Table 2-1. Levels of Significant Exposure to Acrolein – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Kutzman et al. 1984									
58	Rat Dahl (hyper-tension-sensitive) 10 F	62 days 5 days/week 6 hours/day	0, 0.39, 1.40, 3.96	CS, BW, BI, HP, OW	Death			3.96	Increased mortality (100% by day 11)
					Bd wt	1.40			
					Resp		0.39	3.96	LOAEL: Bronchiolar hyperplasia, peripheral lymphoid aggregation, and macrophage clusters SLOAEL: Severe airway epithelial necrosis with massive edema and hemorrhage
					Cardio	1.40			
					Hepatic	1.40			
					Renal	1.40			
					Neuro	1.40			
Leach et al. 1987									
59	Rat (Sprague-Dawley) 10–18 M	3 weeks 5 days/week 6 hours/day	0, 0.17, 1.07, 2.98	BW, OW, GN, HP	Bd wt	1.07	2.98		Decreased body weight at termination (15%)
					Resp		2.98		Nasal squamous metaplasia and degeneration of the respiratory epithelium, neutrophil infiltration, degeneration and atrophy of the olfactory epithelium
					Immuno	2.98			
Liu et al. 2019									
60	Rat (Sprague-Dawley) 36 M	4 weeks 5 days/week 5 hours/day (WB)	0, 3.1	HP	Resp		3.1		Laryngeal epithelial sloughing, cell death, and edema

Table 2-1. Levels of Significant Exposure to Acrolein – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Lyon et al. 1970									
61	Rat (Sprague-Dawley) 7 M, 8 F	6 weeks 5 days/week 8 hours/day	0, 0.7, 3.7	CS, BW, BC, BI, GN, HP, OF	Bd wt	3.7 F 0.7 M		3.7 M	Decreased body weight (21%)
					Resp		0.7		Chronic inflammation in the lungs (peribronchial interstitial infiltration of mononuclear cells) and occasional alveolar distension/ emphysematous changes
					Hemato	3.7			
					Hepatic	3.7			
					Renal	3.7			
Lyon et al. 1970									
62	Rat (Sprague-Dawley) 7–15 M, 8–15 F	90 days 24 hours/day	0, 0.22, 1.0, 1.8	CS, BW, BC, GN, HP	Bd wt	0.22	1 F	1 M	Decreased body weight (11%) Decreased body weight (22%)
					Resp	0.22	1		Occasional pulmonary hemorrhage
					Cardio	1.8			
					Hemato	1.8			
					Hepatic	0.22	1		Focal liver necrosis
					Renal	1.8			
Sherwood et al. 1986									
63	Rat (Sprague-Dawley) 33 M	3 weeks 5 days/week 6 hours/day	0, 0.1, 1.0, 3.0	IX	Immuno	3			

Table 2-1. Levels of Significant Exposure to Acrolein – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Conklin et el. 2017b									
64	Mouse (C57BL/6) 5–15 M	12 weeks 5 days/week 6 hours/day (WB)	0, 0.5, 1	BC, HE, HP, OW	Bd wt	1			
					Resp	1			
					Cardio	1			
					Hemato	0.5	1		Decreased total white blood cell count, neutrophils, lymphocytes, and monocytes
					Musc/skel	1			
					Hepatic	1			
					Renal	1			
					Immuno	1			
					Other noncancer	1			
Feron et al. 1978									
65	Hamster (Golden Syrian) 10 M, 10 F	13 weeks 5 days/week 6 hours/day	0, 0.4, 1.4, 4.9	BW, FI, BC, UR, OW, HP	Bd wt	1.4			
								4.9 F	Decreased body weight (31%)
								4.9 M	Decreased body weight (20%)
					Resp	0.4	1.4		Nasal cavity inflammation
					Cardio	4.9			
					Hemato	1.4 F 4.9 M	4.9 F		Increased number of erythrocytes, lymphocytes, packed cell volume, and hemoglobin content, decreased number of neutrophils
					Hepatic	4.9			
					Renal	4.9			
					Endocr	4.9			
					Immuno	4.9			

Table 2-1. Levels of Significant Exposure to Acrolein – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Neuro	4.9			
					Repro	4.9			
Lyon et al. 1970									
66	Dog (Beagle) 2 M	6 weeks 5 days/week 8 hours/day	0, 0.7, 3.7	CS, BW, BC, BI, GN, HP, OF	Bd wt	3.7			
					Resp		0.7		Chronic inflammation in the lungs (peribronchial interstitial infiltration of mononuclear cells) and occasional alveolar distension/emphysematous changes
					Hemato	3.7			
					Hepatic	3.7			
Lyon et al. 1970									
67	Dog (Beagle) 2–4 M	90 days 24 hours/day	0, 0.22, 1.0, 1.8	CS, BW, BC, HP	Bd wt	1.8			
					Resp		0.22		Moderate emphysema and acute congestion of the lungs; focal vacuolization of the bronchiolar epithelial cells; increased secretory activity; and occasional constriction of bronchioles
					Cardio		0.22		Nonspecific inflammatory changes
					Hemato		0.22		Focal subcapsular hemorrhage of the spleen
					Hepatic		0.22		Nonspecific inflammatory changes
					Renal		0.22		Nonspecific inflammatory changes
					Endocr		0.22		Hyperplasia of the thyroid gland

Table 2-1. Levels of Significant Exposure to Acrolein – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Feron et al. 1978									
68	Rabbit (Dutch) 2 M, 2 F	13 weeks 5 days/week 6 hours/day	0, 0.4, 1.4, 4.9	BW, FI, BC, BI, OW, GN, HP, UR	Bd wt	1.4	4.9		Decreased body weight (12%)
					Resp	1.4	4.9		Nasal lesions (necrotizing rhinitis, neutrophilic infiltration), tracheal lesions (hyperplastic epithelium, mucus cells), lung lesions (bronchitis, hyperplasia, metaplasia)
					Cardio	4.9			
					Hemato	4.9			
					Hepatic	4.9			
					Renal	4.9			
					Endocr	4.9			
					Immuno	4.9			
					Neuro	4.9			
					Repro	4.9			
Lyon et al. 1970									
69	Guinea pig (Hartley) 7 M, 8 F	6 weeks 5 days/week 8 hours/day	0, 0.7, 3.7	BW, OW, HP, OF	Bd wt	3.7			
					Resp		0.7		Chronic inflammation in the lungs (peribrochial interstitial infiltration of mononuclear cells) and occasional emphysema
					Hemato	3.7			
					Hepatic	0.7	3.7		Nonspecific inflammatory changes
					Renal	0.7	3.7		Nonspecific inflammatory changes

Table 2-1. Levels of Significant Exposure to Acrolein – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Lyon et al. 1970									
70	Guinea pig (Hartley) 6–15 M, 8–15 F	90 days 24 hours/day	0, 0.22, 1.0, 1.8	CS, BW, BC, BI, GN, HP	Bd wt	1.8			
					Resp	0.22	1		Pulmonary inflammation (not further described)
					Cardio		0.22		
					Hemato				
					Hepatic		0.22		Nonspecific inflammatory changes
					Renal		0.22		Nonspecific inflammatory changes
CHRONIC EXPOSURE									
Matsumoto et al. 2021									
71	Rat (F344/DuCrlCrlj) 50 M, 50 F	2 years 5 days/week 6 hours/day (WB)	0, 0.1, 0.5, 2	LE, BW, FI, HE, BC, OW, HP	Death			2 F	Increased mortality (32%)
					Bd wt	2 F 0.5 M	2 M		Decreased terminal body weight (12%)
					Resp	0.5	2[c]		Nasal inflammation, metaplasia, eosinophilic changes, and goblet cell hyperplasia (BMCL = 0.012 ppm)
					Hemato	2			
					Hepatic	2			
					Renal	2			
					Dermal	2			
					Endocr	2			
					Immuno	2			
					Repro	2			
					Cancer			2	CEL: Nasal tumors (rhabdomyomas, 8%)

Table 2-1. Levels of Significant Exposure to Acrolein – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Matsumoto et al. 2021									
72	Mouse (B6D2F1/ Crlj) 50 M, 50 F	93 weeks (males); 99 weeks (females) 5 days/week 6 hours/day (WB)	0, 0.1, 0.4, 1.6	LE, BW, FI, HE, BC, OW, HP	Bd wt	1.6 F 0.4 M	1.6 M		Decreased terminal body weight (17%)
					Resp	0.1 F	0.4 F		Nasal inflammation, hyperplasia, metaplasia, and regeneration
						0.4 M	1.6 M		Nasal inflammation, hyperplasia, metaplasia, and regeneration
					Hemato	1.6			
					Hepatic	1.6			
					Renal	1.6			
					Dermal	1.6			
					Endocr	1.6			
					Immuno	1.6			
					Repro	1.6			
					Cancer			1.6	CEL: Nasal tumors (adenomas, 32%)
Feron and Kruysse 1977									
73	Hamster (Golden Syrian) 18 M, 18 F	52 weeks 5 days/week 7 hours/day	0, 4.0	CS, BW, BC, BI, GN, OW, HP	Bd wt		4 F		Decreased body weight (10%)
							4 M		Decreased body weight (11%)
					Resp		4		Nasal inflammation and epithelial metaplasia, neutrophilic infiltrates, and submucosa thickening
					Cardio	4			
					Hemato	4 M	4 F		Increased hemoglobin content and packed cell volume

Table 2-1. Levels of Significant Exposure to Acrolein – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic	4			
					Renal	4			
					Immuno	4			
					Neuro	4			
					Repro	4			

Green shading indicates studies selected for derivation of inhalation MRLs.

[a]The number corresponds to entries in Figure 2-2; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-2. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive a provisional acute-duration inhalation MRL of 0.003 ppm based on nose and throat irritation and decreased respiratory rate. See Appendix A for more detailed information regarding the MRL.
[c]Used to derive a provisional chronic-duration inhalation MRL of 0.0004 ppm based on nasal respiratory gland metaplasia. This MRL was also considered protective for intermediate-duration exposure and adopted for the intermediate-duration inhalation MRL. See Appendix A for more detailed information regarding the MRL.

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; B = both males and females; BC = blood chemistry; BI = biochemical changes; Bd wt or BW = body weight; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; F = female(s); FI = food intake; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; IX = immune function; LC_{50} = median lethal concentration; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skeletal = muscular/skeletal; (N) = nose-only; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; NX = neurological function; OF = organ function; OW = organ weight; RD_{50} = exposure concentration producing a 50% respiratory rate decrease; Repro = reproductive; Resp = respiratory; RX = reproductive function; SLOAEL = serious lowest-observed-adverse-effect level; UR = urinalysis; (WB) = whole body

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

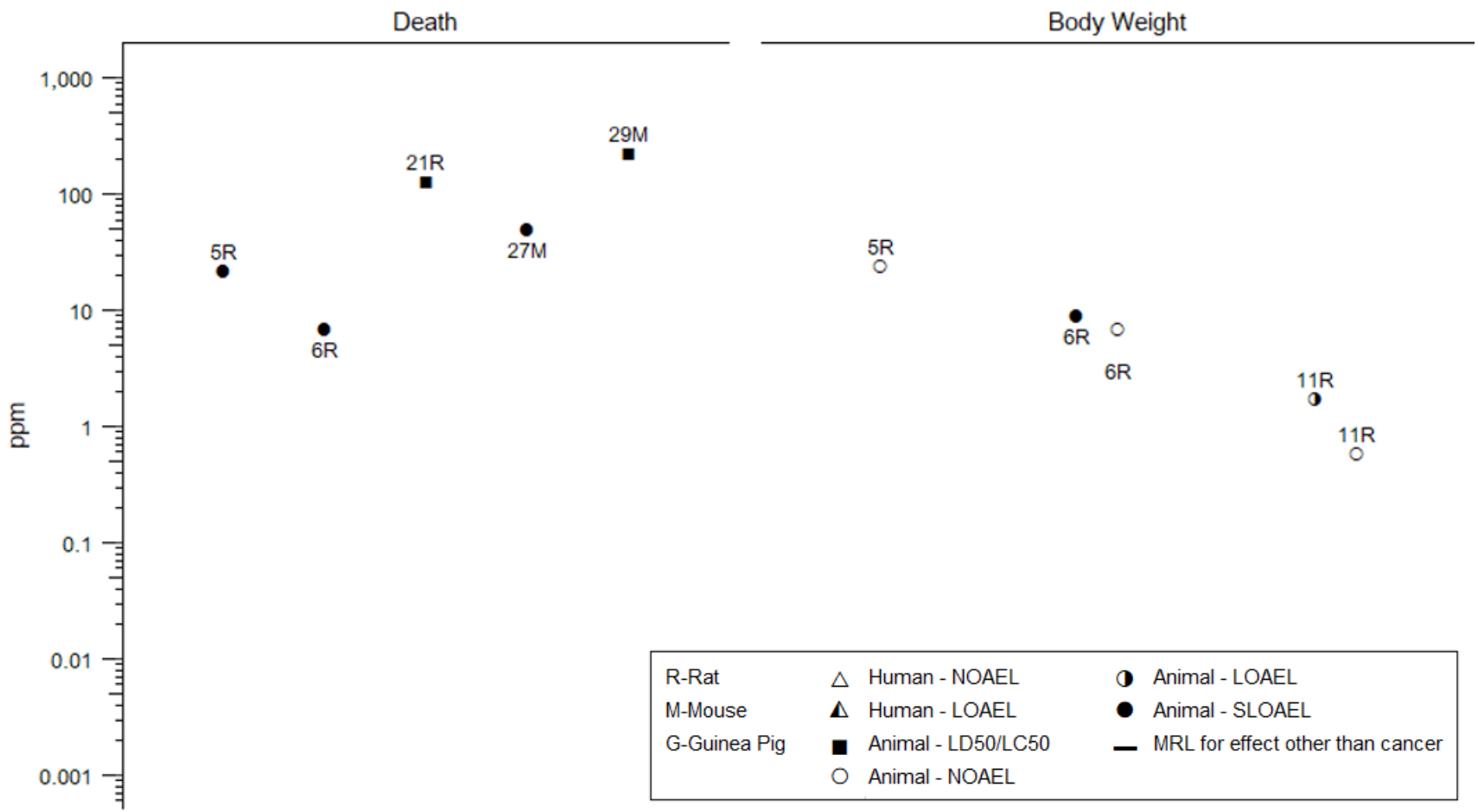

Figure 2-2. Levels of Significant Exposure to Acrolein – Inhalation
Acute (≤14 days)

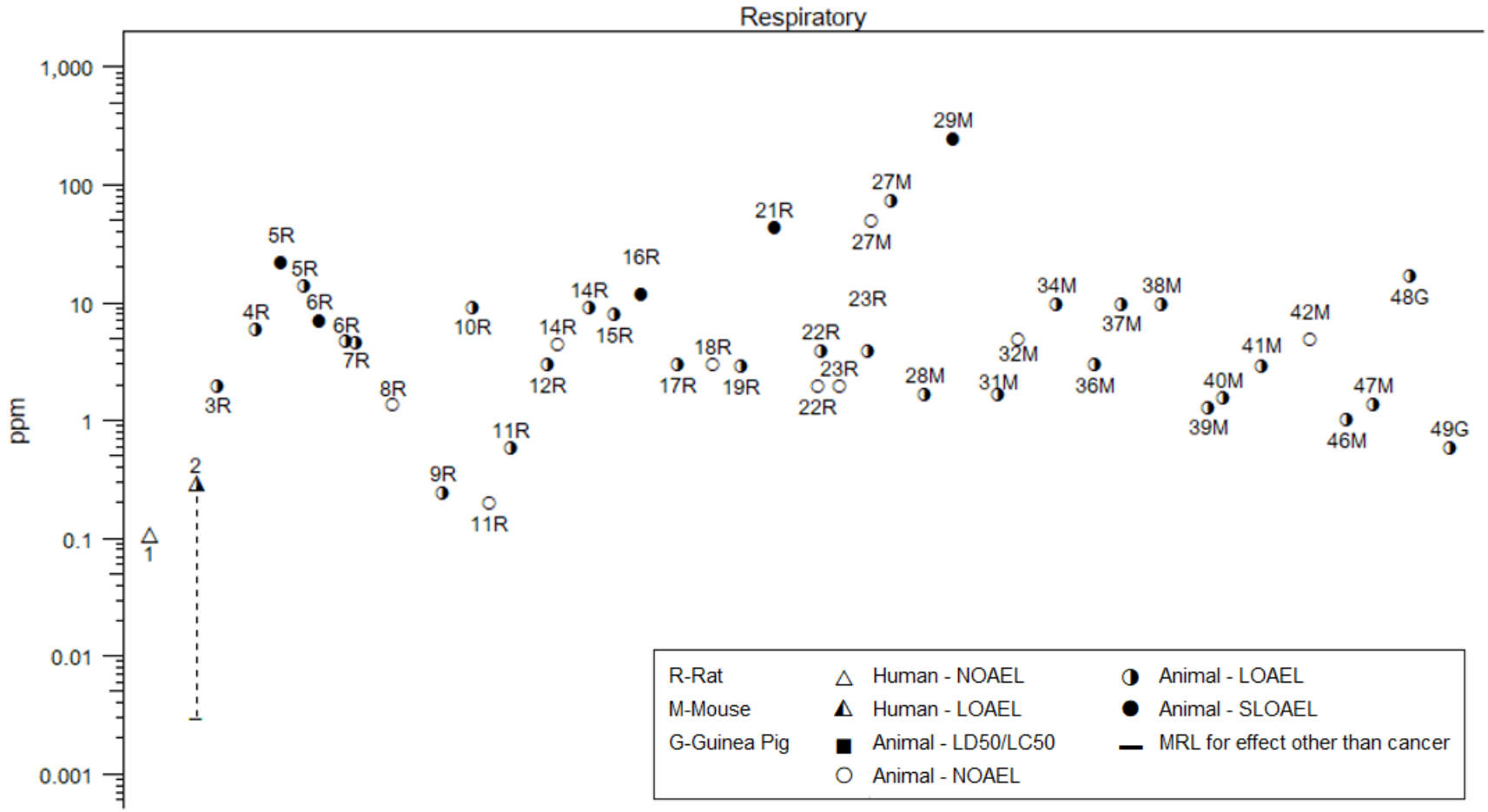

Figure 2-2. Levels of Significant Exposure to Acrolein – Inhalation
Acute (≤14 days)

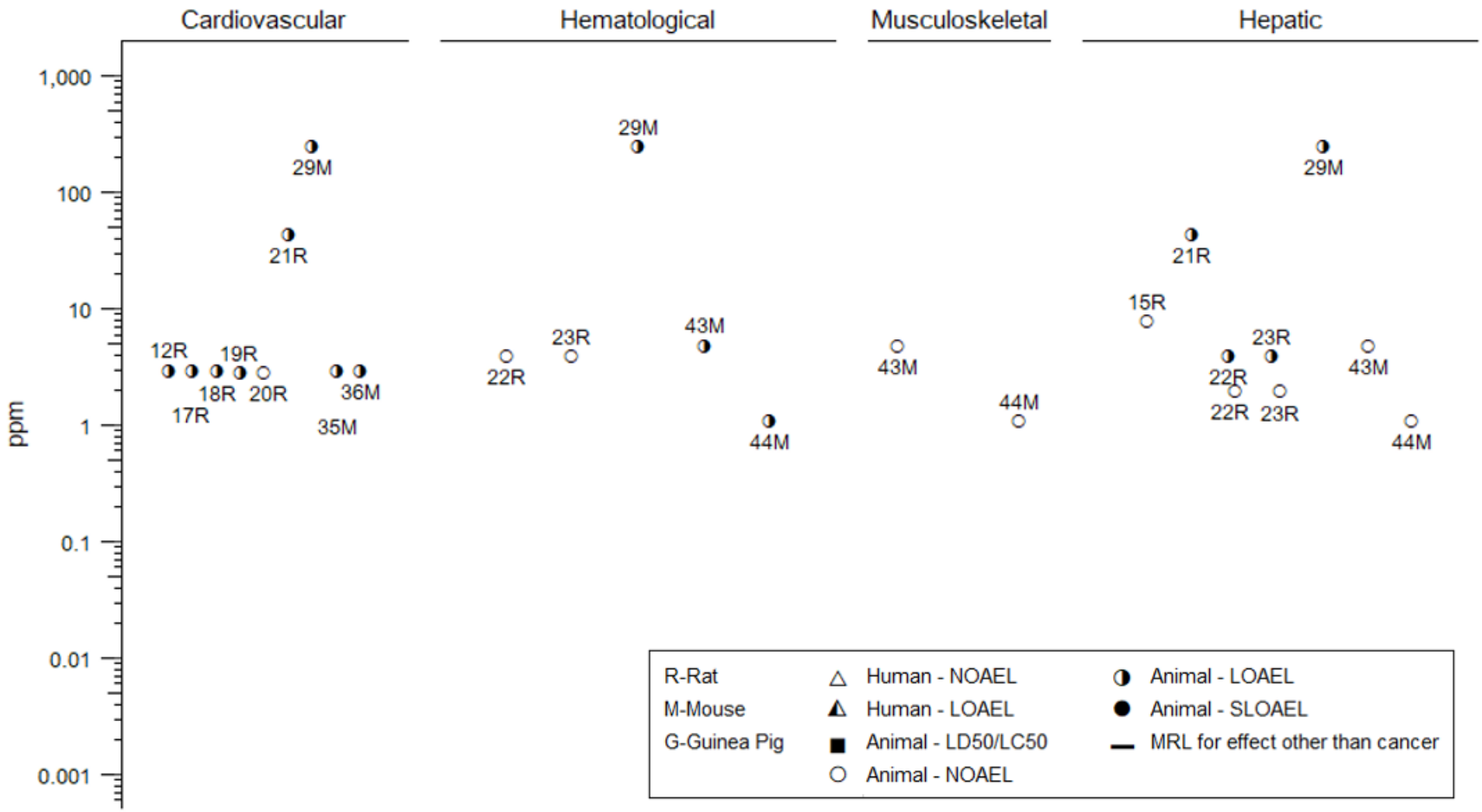

Figure 2-2. Levels of Significant Exposure to Acrolein – Inhalation
Acute (≤14 days)

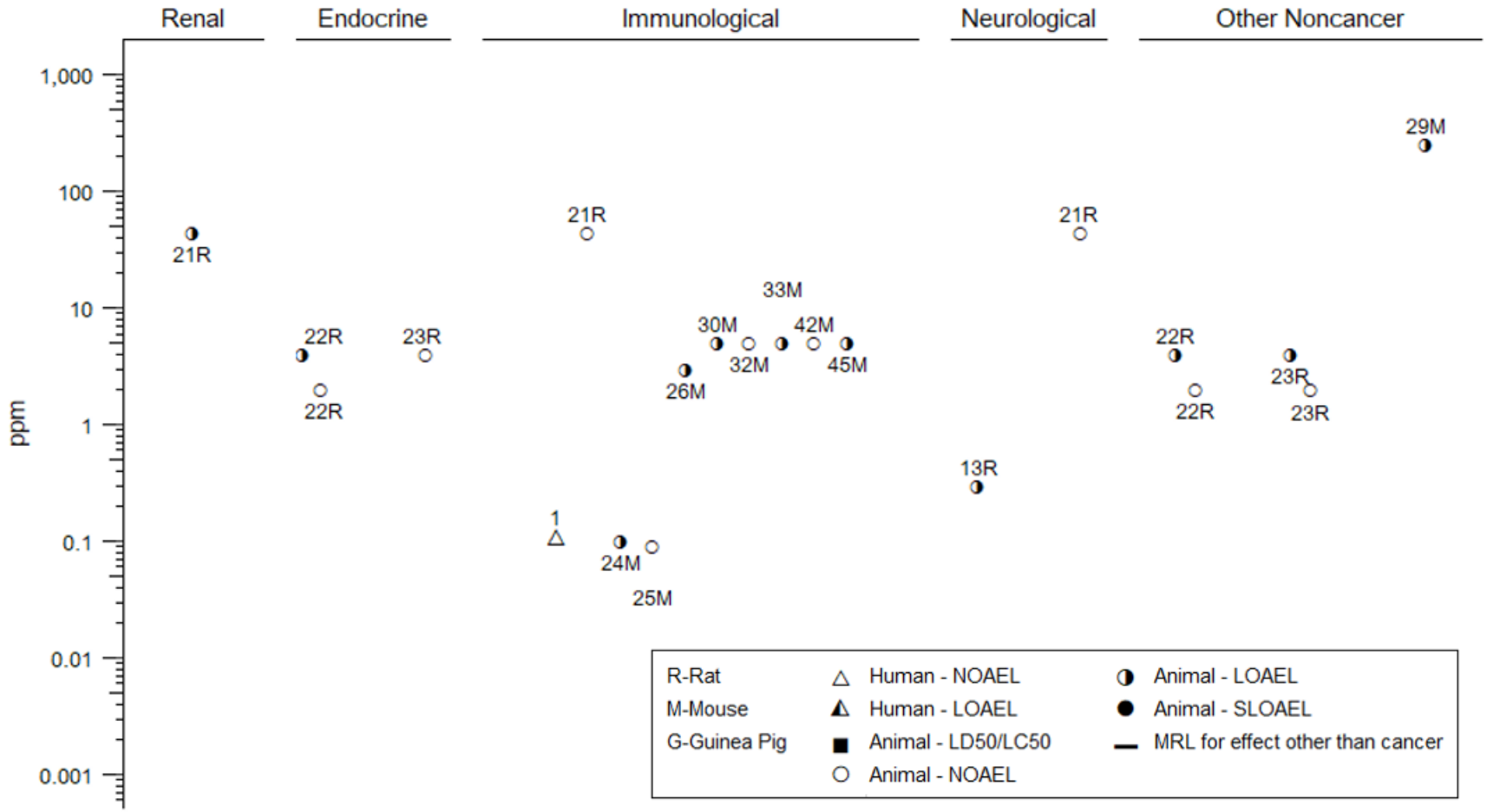

Figure 2-2. Levels of Significant Exposure to Acrolein – Inhalation
Intermediate (15–364 days)

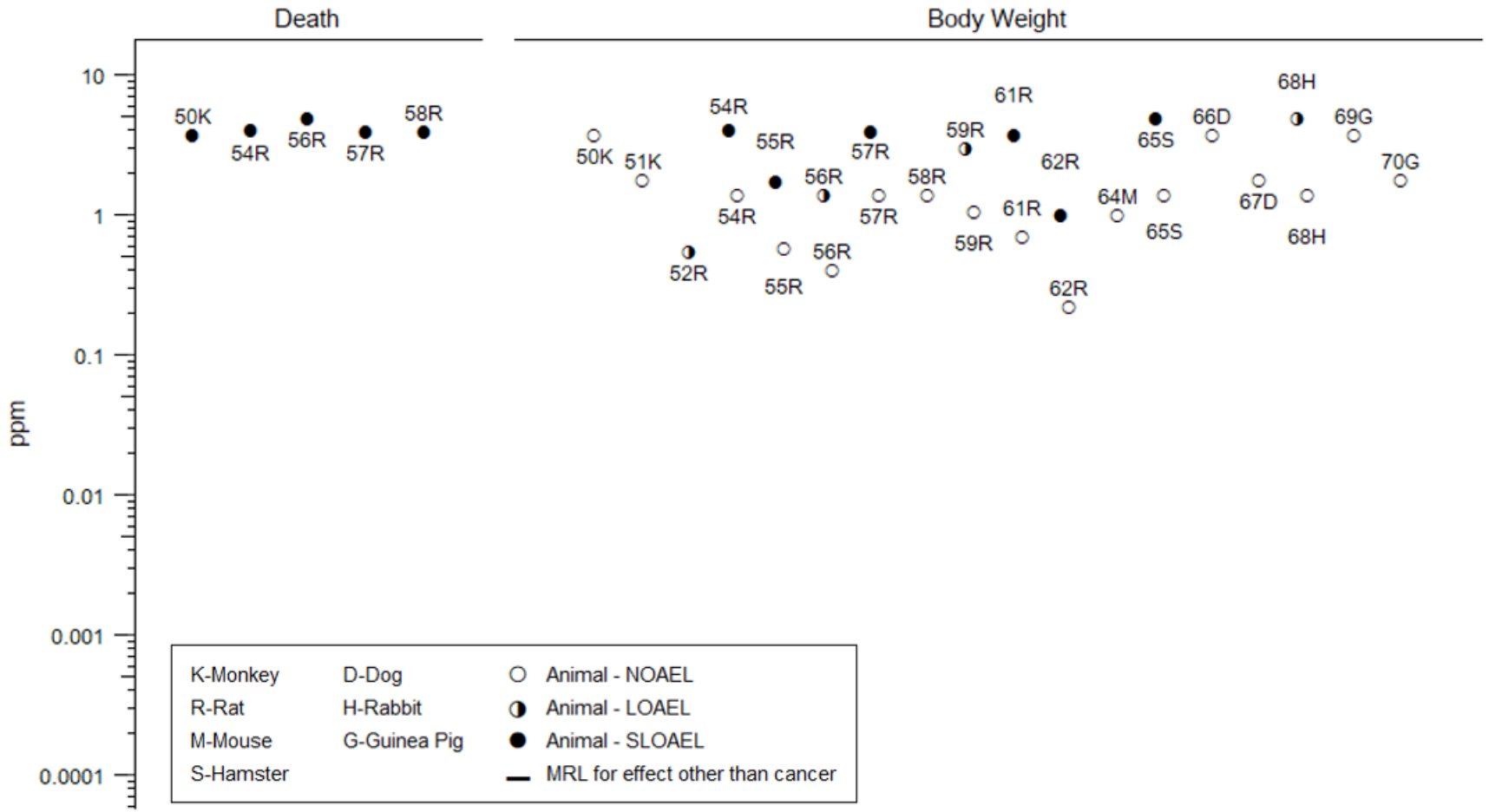

Figure 2-2. Levels of Significant Exposure to Acrolein – Inhalation
Intermediate (15–364 days)

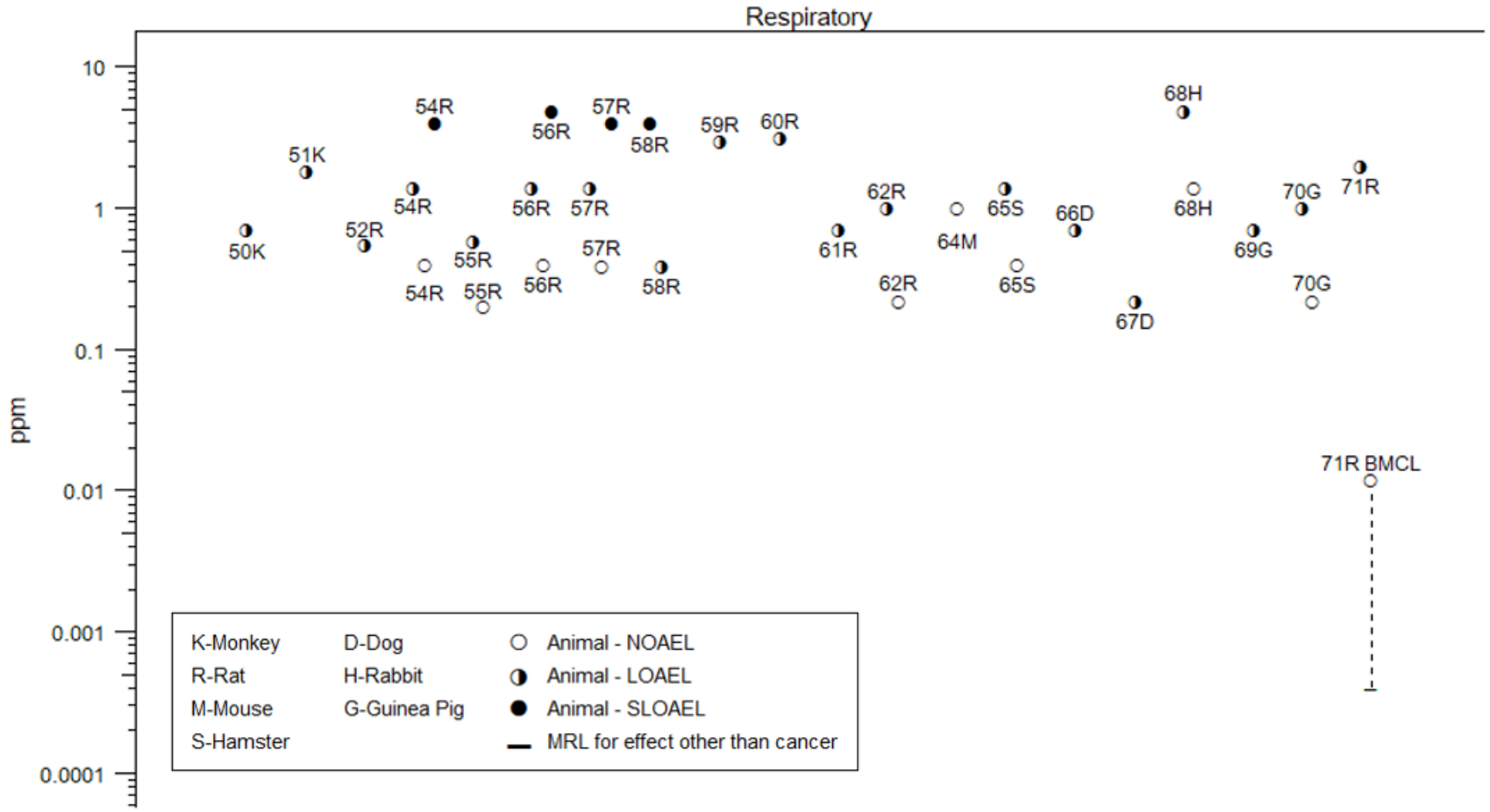

Figure 2-2. Levels of Significant Exposure to Acrolein – Inhalation
Intermediate (15–364 days)

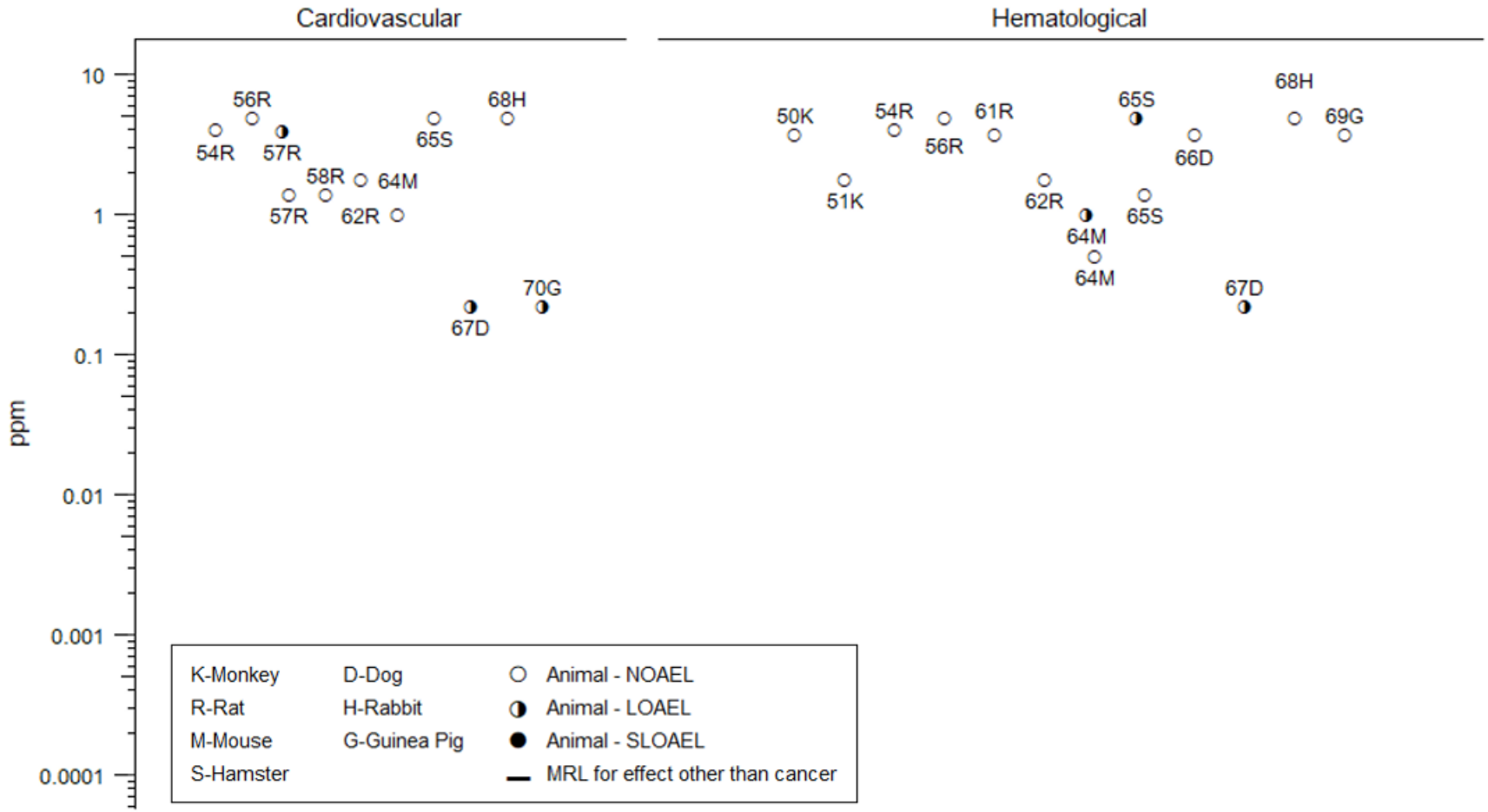

Figure 2-2. Levels of Significant Exposure to Acrolein – Inhalation
Intermediate (15–364 days)

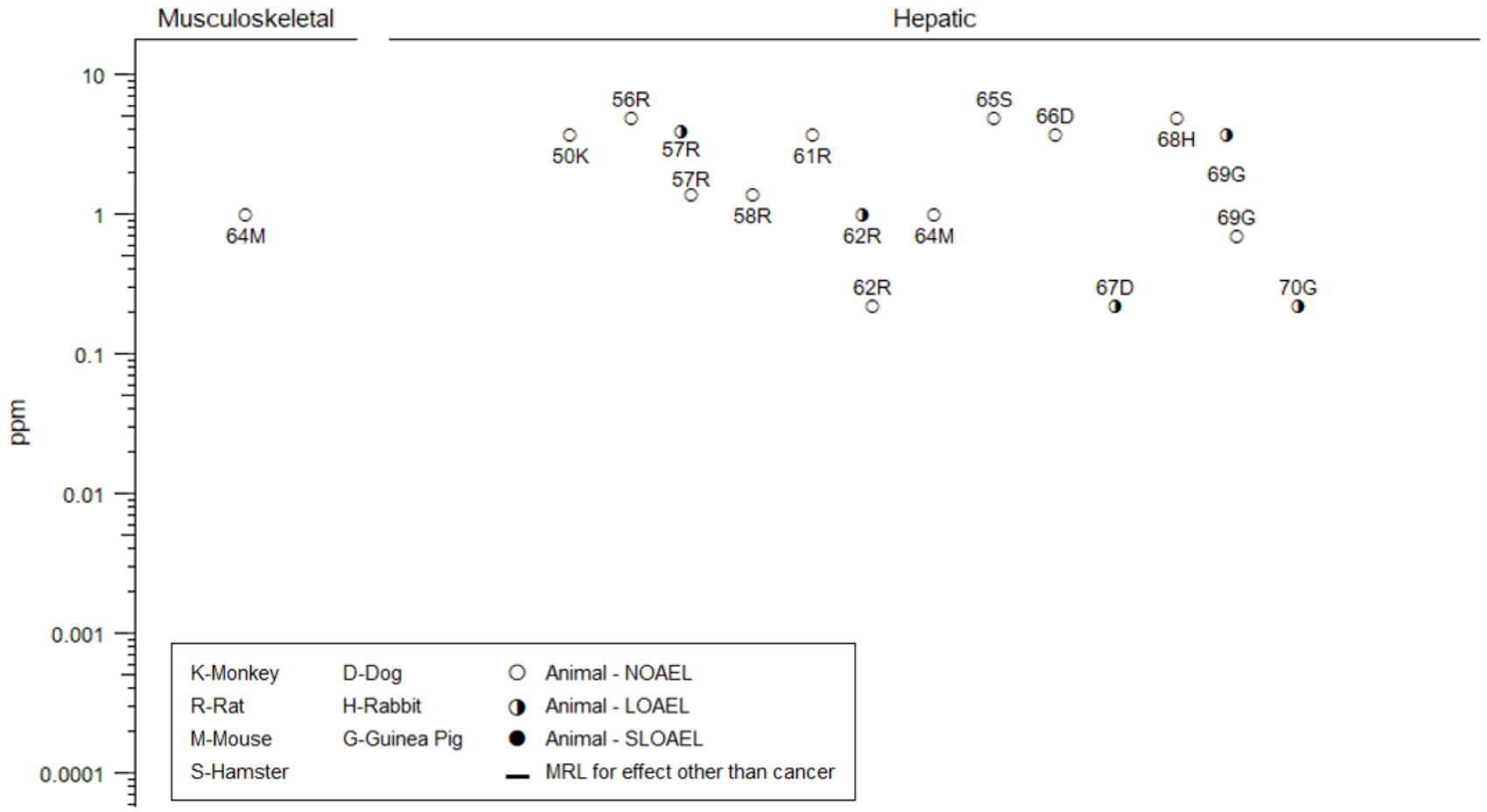

Figure 2-2. Levels of Significant Exposure to Acrolein – Inhalation
Intermediate (15–364 days)

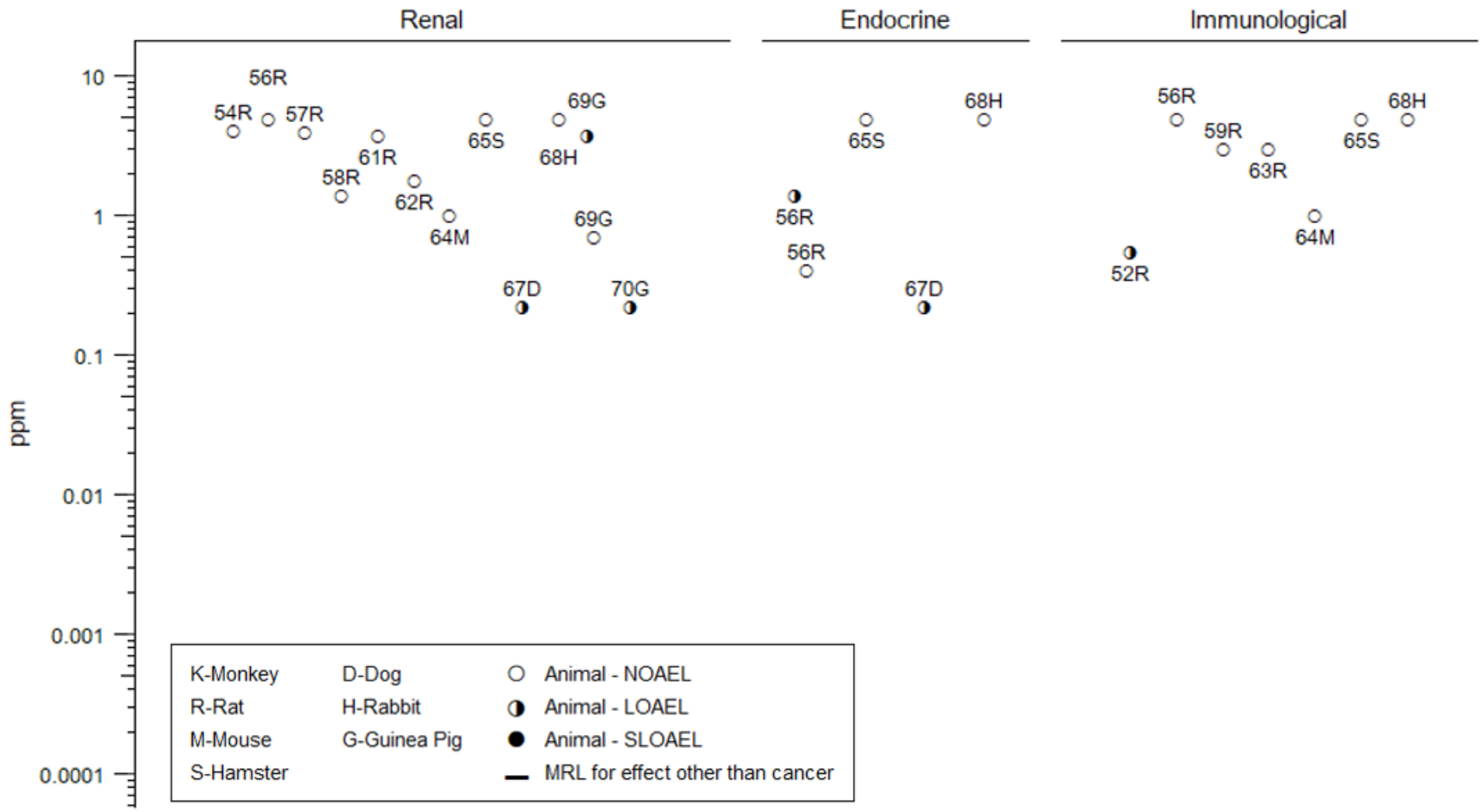

Figure 2-2. Levels of Significant Exposure to Acrolein – Inhalation
Intermediate (15–364 days)

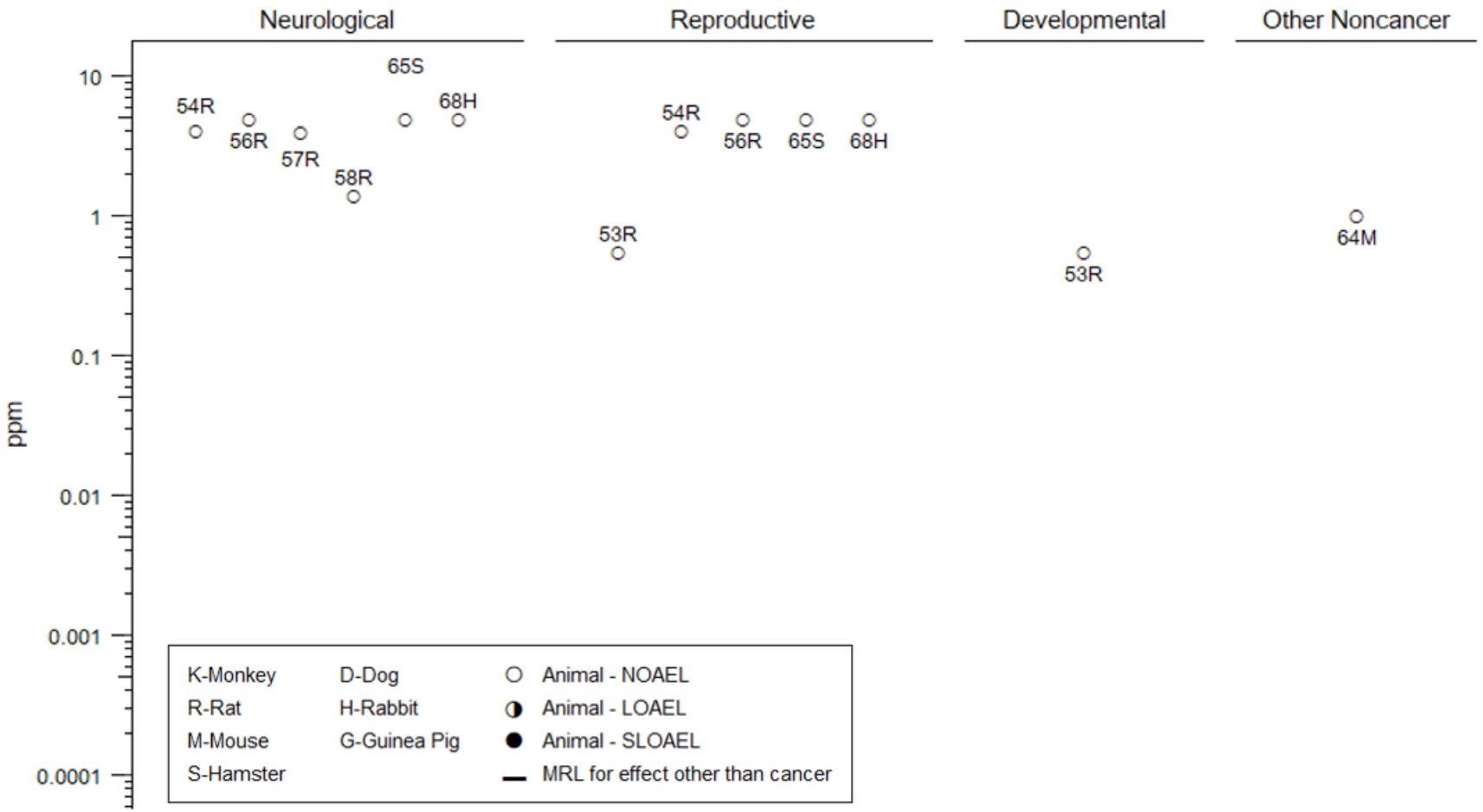

Figure 2-2. Levels of Significant Exposure to Acrolein – Inhalation
Chronic (≥365 days)

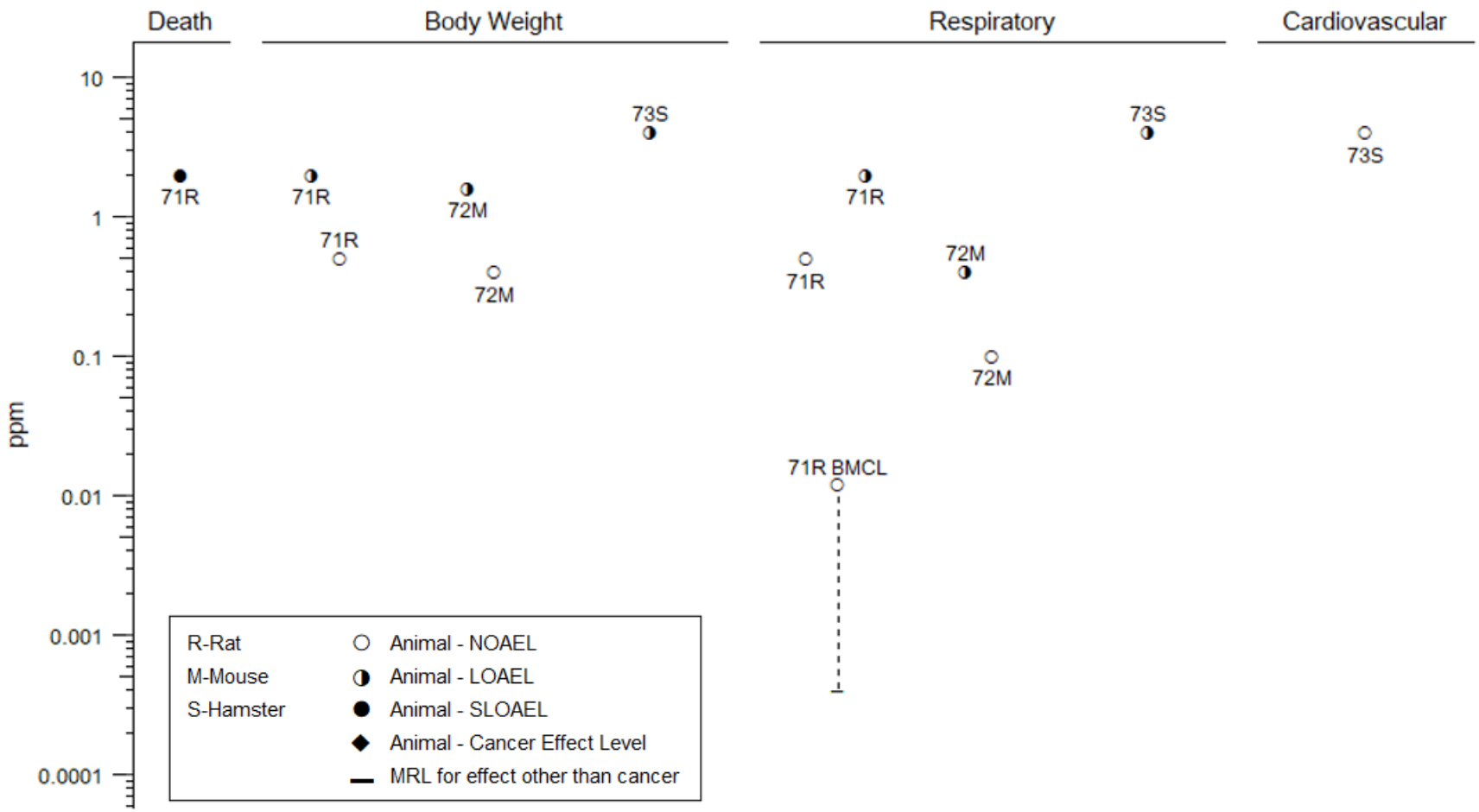

Figure 2-2. Levels of Significant Exposure to Acrolein – Inhalation
Chronic (≥365 days)

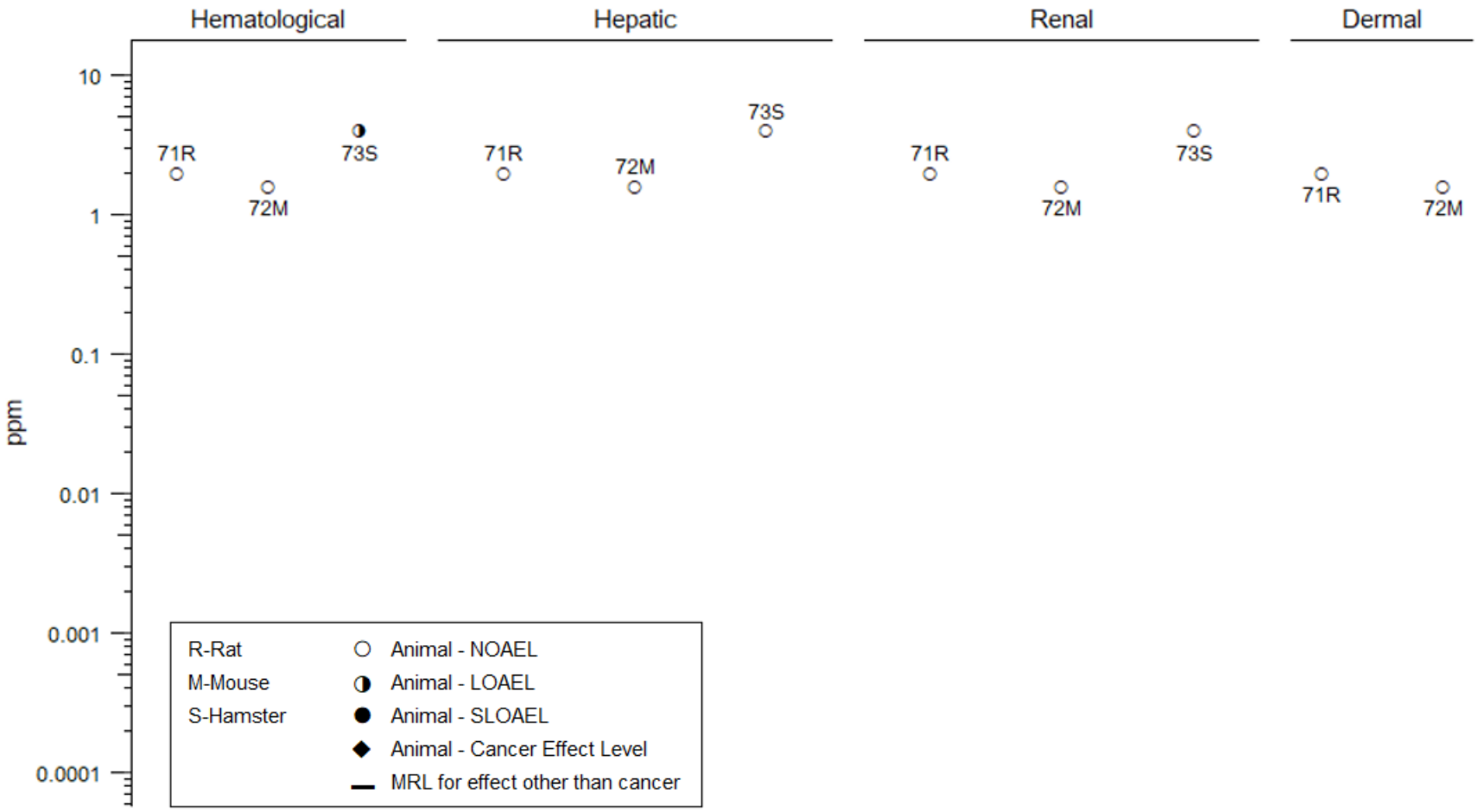

Figure 2-2. Levels of Significant Exposure to Acrolein – Inhalation
Chronic (≥365 days)

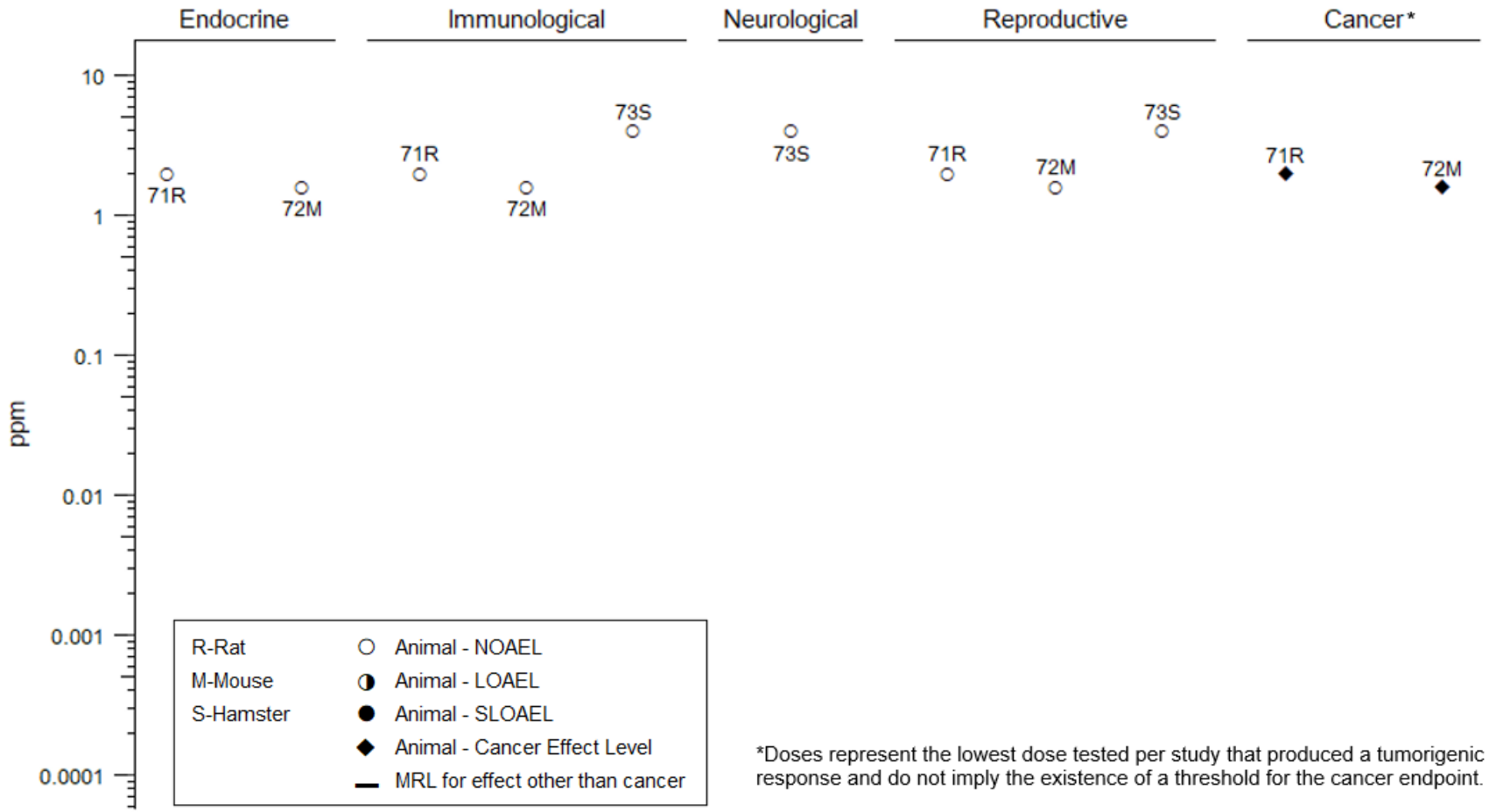

Table 2-2. Levels of Significant Exposure to Acrolein – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Draminski et al. 1983									
1	Rat (Wistar) 10 F	Once (GO)	10		Death			10	LD_{50}
EPA 1983									
2	Rat (Sprague-Dawley) 40 F	13 days GDs 7–19 (GW)	0, 3.6, 6.0, 10.0	CS, BW, OW, GN, DX	Death			10	Increased mortality (30%)
					Bd wt	3.6		6	Decreased extra-gestational body weight gain (36%) (maternal weight gain minus gravid uterine weight)
					Develop	6		10	Increased incidence of skeletal abnormalities
Sprince et al. 1979									
3	Rat (CFE) 40 M	Once (G)	0, 11.2	CS	Death			11.2	Increased mortality (38/40)
					Neuro		11.2		Loss of elevation reflexes, poor body tone, and loss of tail-pinch response
Conklin et al. 2010									
4	Mouse (C57BL/6J) 3–9 M	Once (GW)	0, 0.1, 0.5, 1.0, 2.0, 5.0	BW, HE, BC, GN, OW, HP	Bd wt	5			
					Hemato	5			
					Hepatic	2	5		Increased plasma cholesterol, phospholipids, and triglycerides
					Renal	5			
Sithu et al. 2010									
5	Mouse C57BL/6J) 8 M	Single administration	0, 1, 2, 5	HE	Hemato		5		Platelet aggregation

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Parent et al. 1993									
6	Rabbit (New Zealand) 20 F	13 days GDs 7–19 (GW)	0, 0.1, 0.75, 2.0	CS, BW, GN, OW, DX	Bd wt	0.75	2		Body weight loss (80 versus 0 g in controls)
					Develop	2			
INTERMEDIATE EXPOSURE									
Auerbach et al. 2008; NTP 2006a									
7	Rat (Fischer-344) 10 M, 10 F	14 weeks 5 days/week (GW)	0, 0.75, 1.25, 2.5, 5, 10	BW	Death			10	Increased mortality (80%)
					Bd wt	5			
							10 F		Decreased body weight (10%)
								10 M	Decreased body weight (22%)
					Resp	5	10		Abnormal breathing, nasal inflammation
					Cardio	10			
					Gastro			10	Glandular stomach hemorrhage, necrosis, inflammation
						1.25 F	2.5 F		Forestomach squamous epithelial hyperplasia
						2.5 M	5 M		Forestomach squamous epithelial hyperplasia
					Hemato	2.5	5		Increased reticulocyte and platelet counts
					Musc/skel	10			
					Hepatic	10			
					Renal	10			
					Dermal	10			
					Ocular	10			
					Endocr	10			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Immuno	5		10	Decreased thymus weight, thymocyte atrophy and necrosis, lymphoid follicular cell depletion in the spleen
					Neuro	10			
					Repro	10			
Huang et al. 2013									
8	Rat (Sprague-Dawley) 15 M	8 weeks (GW)	0, 2.5	CS, BW, HP, NX	Bd wt	2.5			
					Cardio	2.5			
					Neuro		2.5		Increased escape latency (Morris water maze), neuronal loss and inflammation in the hippocampus
Parent et al. 1992c									
9	Rat (Sprague-Dawley) 30 M, 30 F	140 days 2 generations (GW)	0, 1, 3, 6	CS, BW, FI, DX	Death			6	Increased mortality (20% in F0, 19% in F1)
					Bd wt	6			
					Resp	3		6	Breathing difficulties (rales, labored breathing, gasping, hyperpnea)
					Cardio	6			
					Gastro	3		6	Stomach ulcers, erosion of the glandular mucosa, and hyperplasia in the forestomach
					Hepatic	6			
					Renal	6			
					Endocr	6			
					Immuno	6			
					Neuro	6			
					Repro	6			

Table 2-2. Levels of Significant Exposure to Acrolein – 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		Decreased pup weight (7% at PND 21 in F1 generation)
Auerbach et al. 2008; NTP 2006a									
10	Mouse (B6C3F1) 10 M, 10 F	14 weeks 5 days/week (GW)	0, 1.25, 2.5, 5, 10, 20	BW	Death			20	Increased mortality (100%)
					Bd wt	10			
					Resp	20			
					Cardio	20			
					Gastro			20	Glandular stomach hemorrhage, epithelial necrosis, and chronic active inflammation
						2.5 F	5 F		Forestomach squamous epithelial hyperplasia
						1.25 M	2.5 M[b]		Forestomach squamous epithelial hyperplasia (BMDL = 0.22 mg/kg/day)
					Hemato	10			
					Musc/skel	20			
					Hepatic	20			
					Renal	20			
					Dermal	20			
					Ocular	20			
					Endocr	20			
					Immuno	10		20	Necrosis in the mandibular and mesenteric lymph node, depletion of the lymphoid follicle in the spleen, necrosis in the thymus
					Neuro	20			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Repro	20			
Chen et al. 2019									
11	Mouse (ICR) 4-6 M	4 weeks (GW)	0, 2.5, 5	CS, BW, HP	Bd wt		2.5		Decreased body weight (15%)
					Musc/skel		2.5		Decreased soleus muscle weight and cross-sectional area
					Neuro		2.5		Decreased rotarod latency
Ismahil et al. 2011									
12	Mouse (C57BL/6J) 8–16 M	48 days (GW)	0, 1	LE, CS, BW, HP, OF	Bd wt	1			
					Cardio		1		Myocardial inflammation, myocyte hypertrophy and cell death, left ventricle remodeling and dysfunction
Wang et al. 2021									
13	Mouse (ICR) 8 M	4 weeks (GW)	0, 2.5, 5	BI	Other noncancer		2.5		Increased blood glucose and insulin, impaired glucose tolerance
CHRONIC EXPOSURE									
Parent et al. 1992a									
14	Rat (Sprague-Dawley) 50 M, 50 F	102 weeks (GW)	0, 0.05, 0.5, 2.5	CS, BW, FI, HE, BC, UR, OP, OW, GN	Death			0.5 F	Increased mortality
					Bd wt	2.5			
					Resp	2.5			
					Cardio	2.5			
					Gastro	2.5			
					Hemato	2.5			
					Musc/skel	2.5			
					Hepatic	2.5			
					Renal	2.5			
					Dermal	2.5			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Ocular	2.5			
					Endocr	2.5			
					Immuno	2.5			
					Neuro	2.5			
					Repro	2.5			
Parent et al. 1991a									
15	Mouse (CD-1) 70–75 M, 70–75 F	18 months (GW)	0, 0.5, 2.0, 4.5	BW, OW, FI, GN, HP, HE	Death			4.5 M	Increased mortality (28%)
					Bd wt	4.5			
					Resp	4.5			
					Cardio	4.5			
					Gastro	4.5			
					Hemato	4.5			
					Musc/skel	4.5			
					Hepatic	4.5			
					Renal	4.5			
					Dermal	4.5			
					Ocular	4.5			
					Endocr	4.5			
					Immuno	4.5			
					Neuro	4.5			
					Repro	4.5			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Parent et al. 1992b									
16	Dog (Beagle) 24 M, 24 F	53 weeks (C)	0, 0.1, 0.5, 1.5–2.0	BC, BW, CS, GN, HP, HE, UR, OW	Bd wt	2			
					Resp	2			
					Cardio	2			
					Gastro	0.1	0.5		Vomiting
					Hemato	2			
					Musc/skel	2			
					Hepatic	2			
					Renal	2			
					Dermal	2			
					Ocular	2			
					Endocr	2			
					Immuno	2			
					Neuro	2			
					Repro	2			

Green shading indicates the study selected for derivation of oral MRL.

[a]The number corresponds to entries in Figure 2-3; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-3. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive a provisional intermediate-duration oral MRL of 0.002 mg/kg/day based on forestomach squamous epithelial hyperplasia. See Appendix A for more detailed information regarding the MRL.

BC = blood chemistry; BI = biochemical changes; Bd wt or BW = body weight; (C) = capsule; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; F = female(s); FI = food intake; (G) = gavage; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; (GO) = gavage in oil; (GW) = gavage in water; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; LD_{50} = median lethal dose; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skeletal = muscular/skeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NX = neurological function; OF = organ function; OP = ophthalmology; OW = organ weight; PND = postnatal day; Repro = reproductive; Resp = respiratory; RX = reproductive function; UR = urinalysis

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

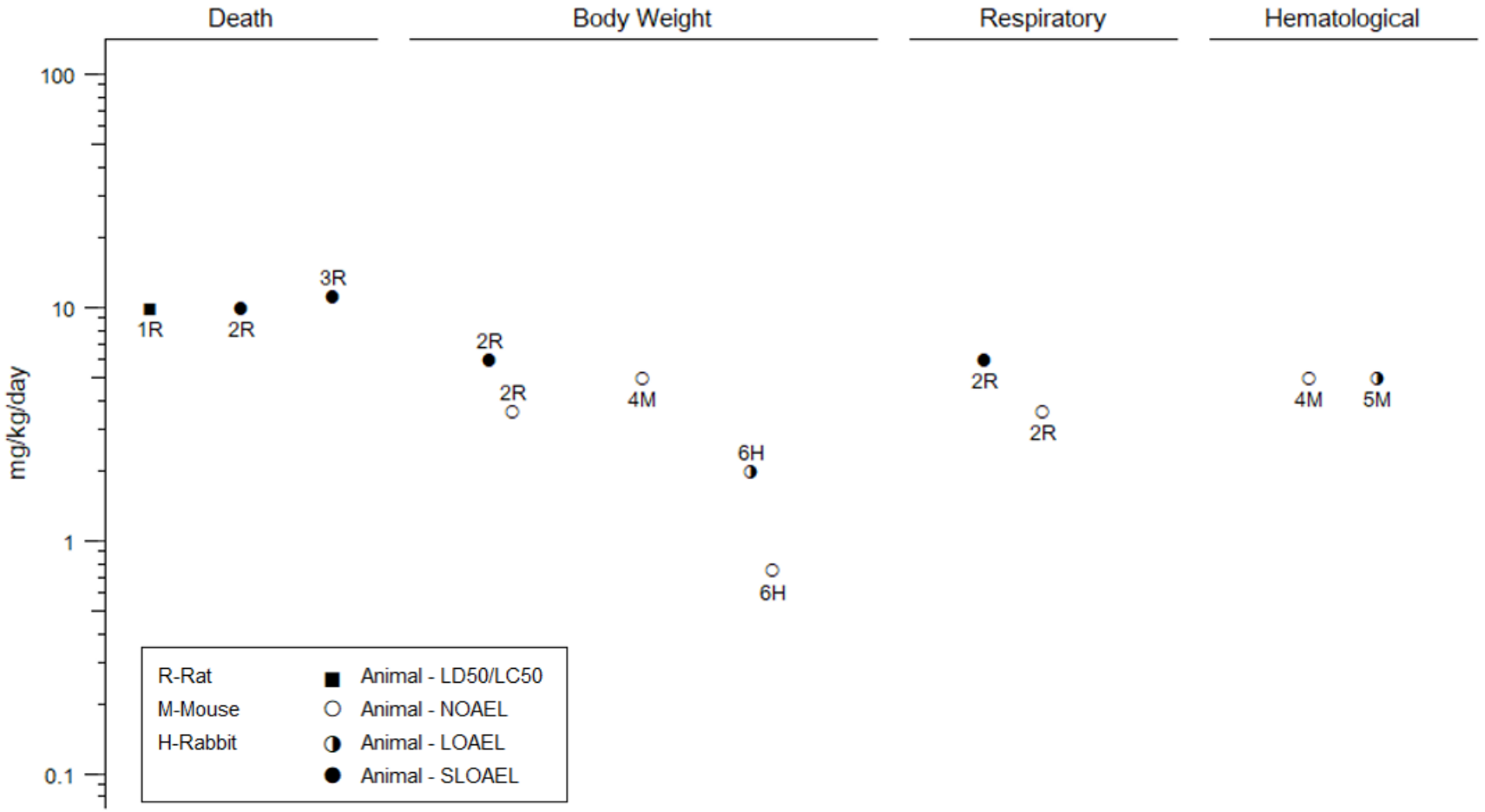

Figure 2-3. Levels of Significant Exposure to Acrolein – Oral
Acute (≤14 days)

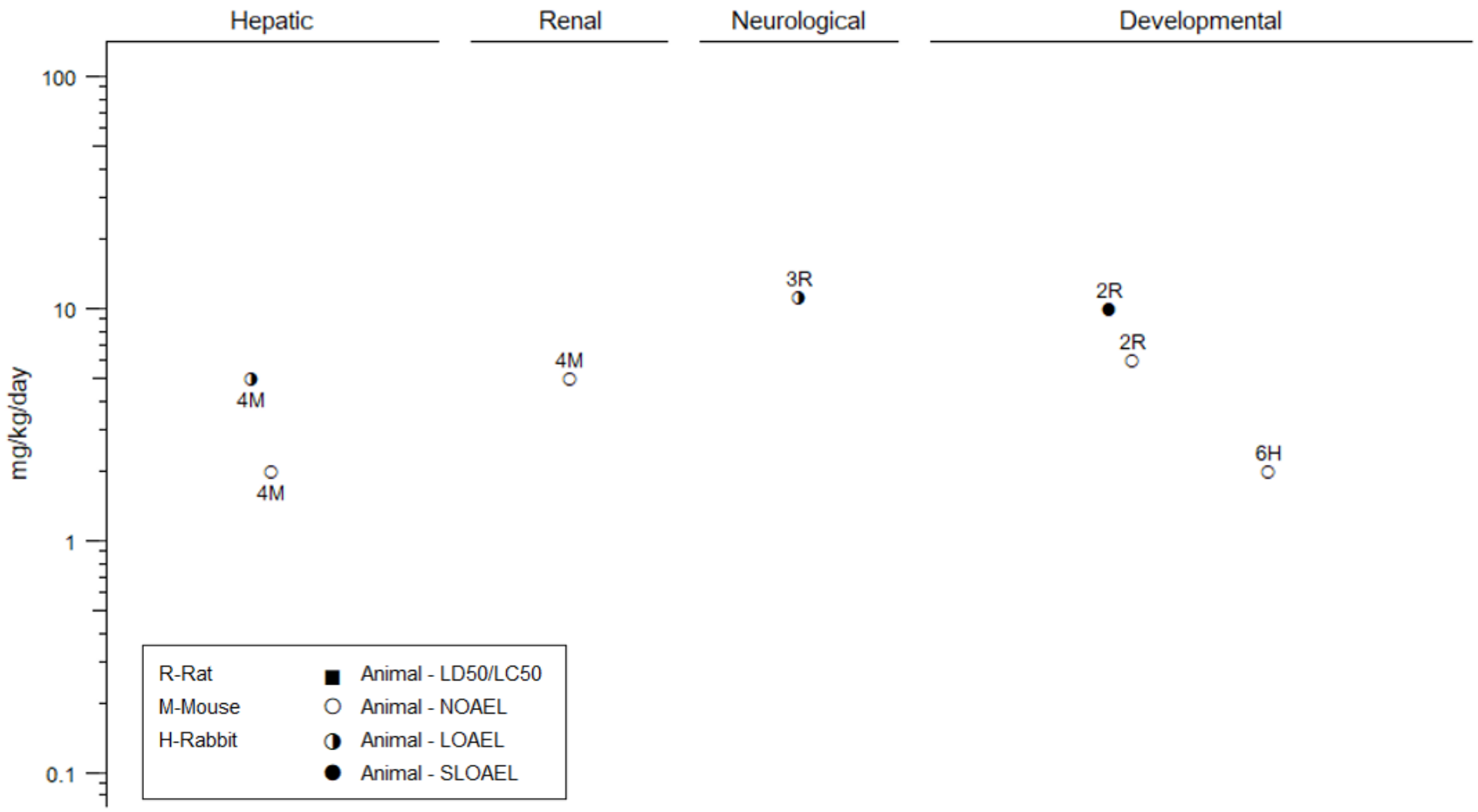

Figure 2-3. Levels of Significant Exposure to Acrolein – Oral
Intermediate (15–364 days)

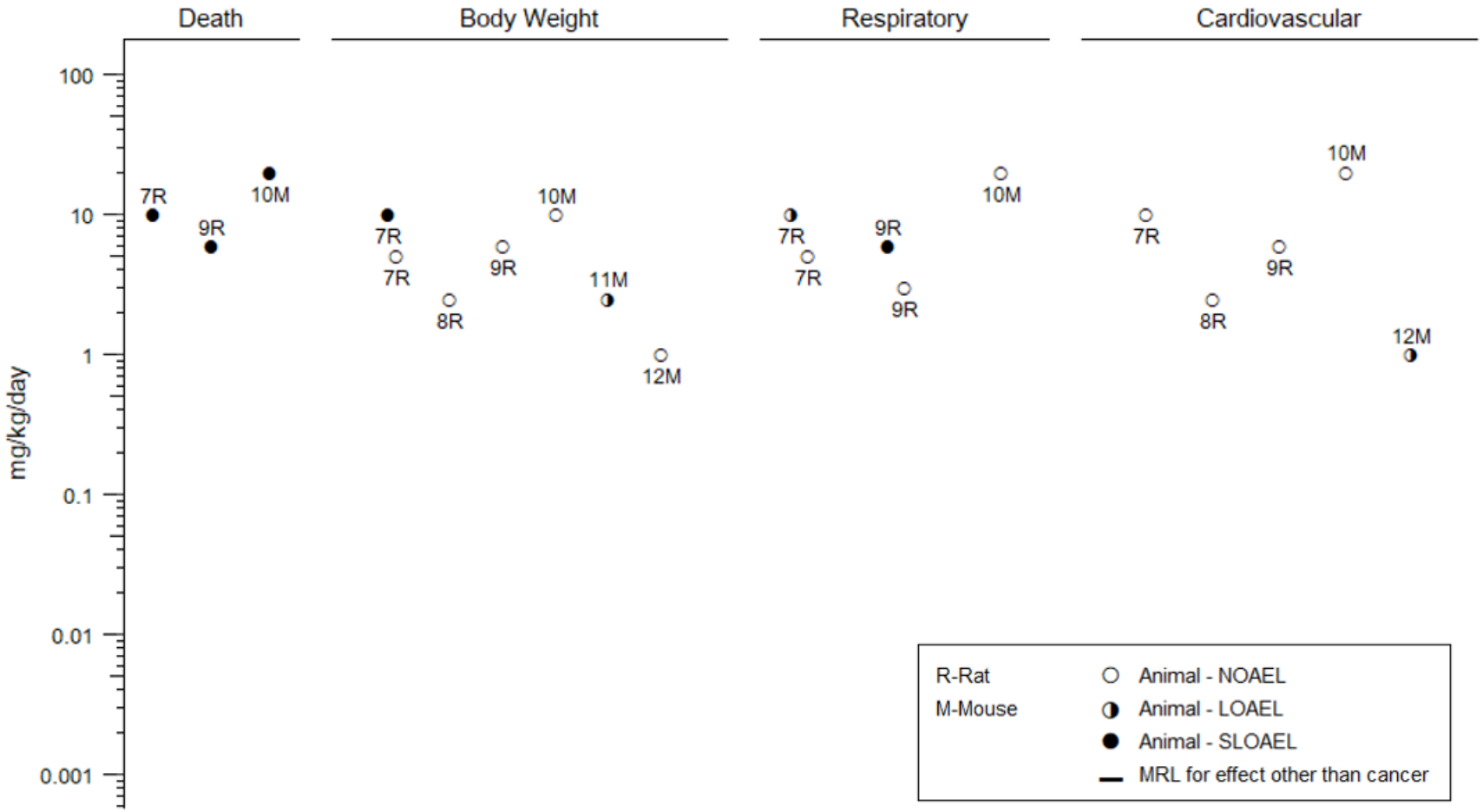

Figure 2-3. Levels of Significant Exposure to Acrolein – Oral
Intermediate (15–364 days)

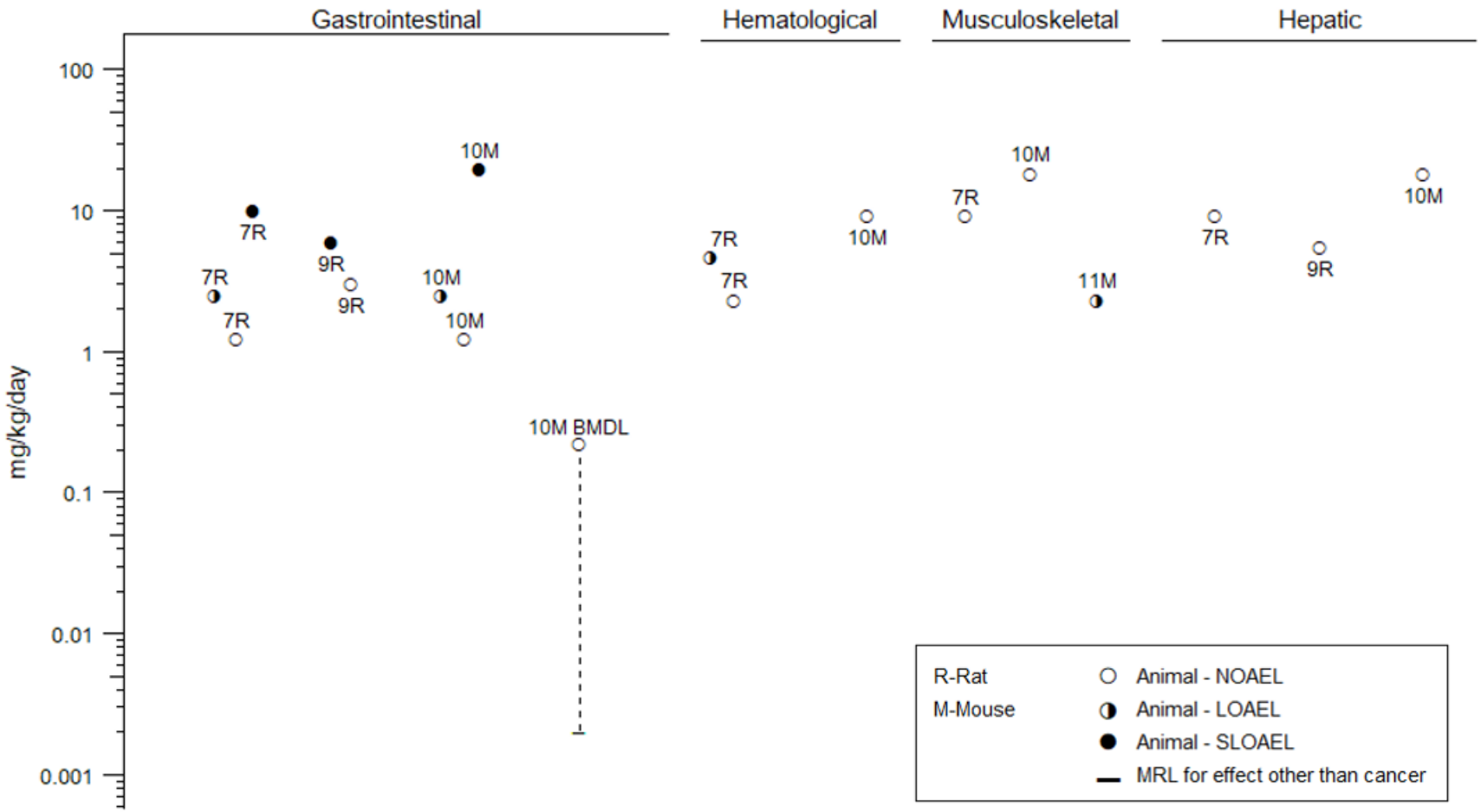

Figure 2-3. Levels of Significant Exposure to Acrolein – Oral
Intermediate (15–364 days)

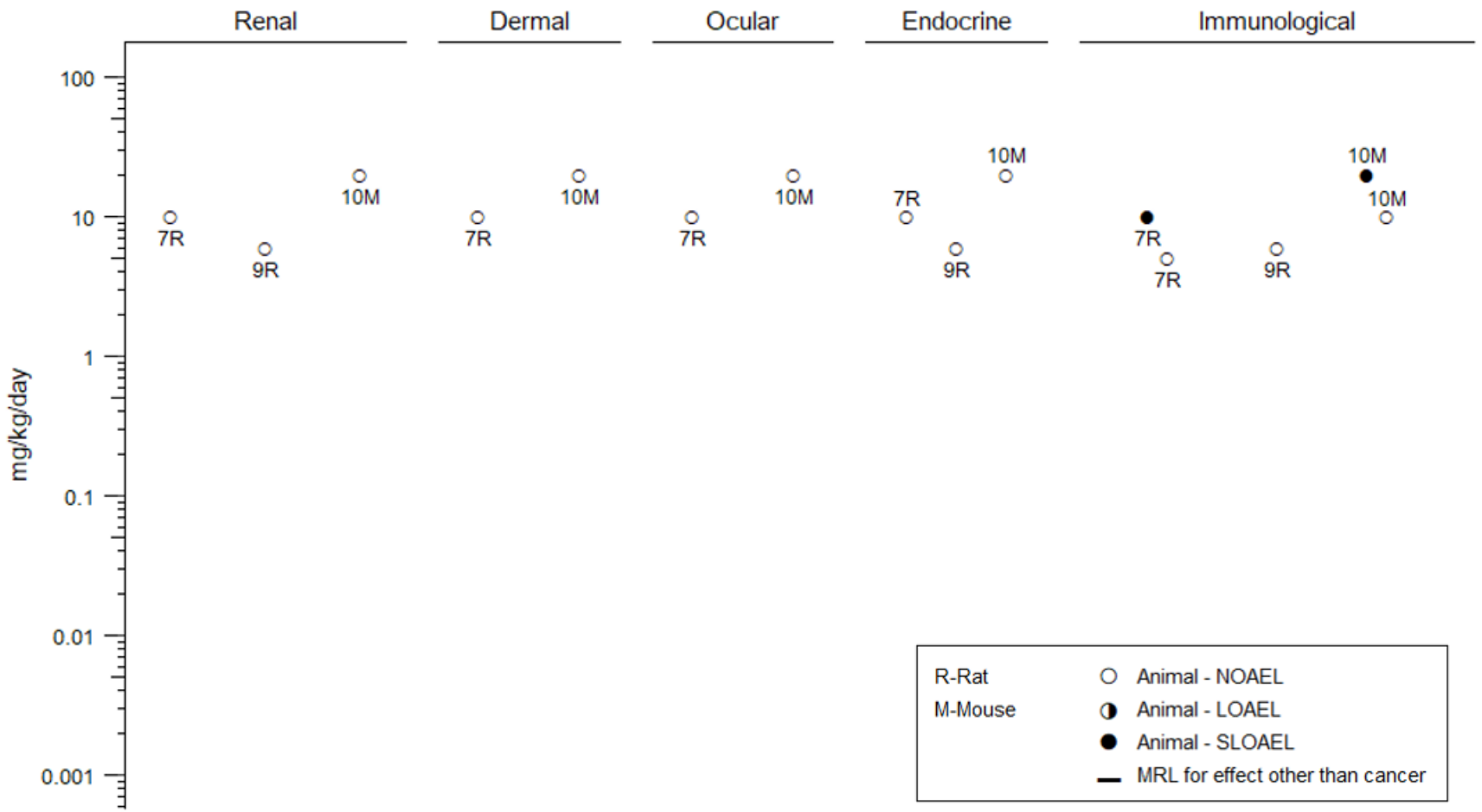

Figure 2-3. Levels of Significant Exposure to Acrolein – Oral
Intermediate (15–364 days)

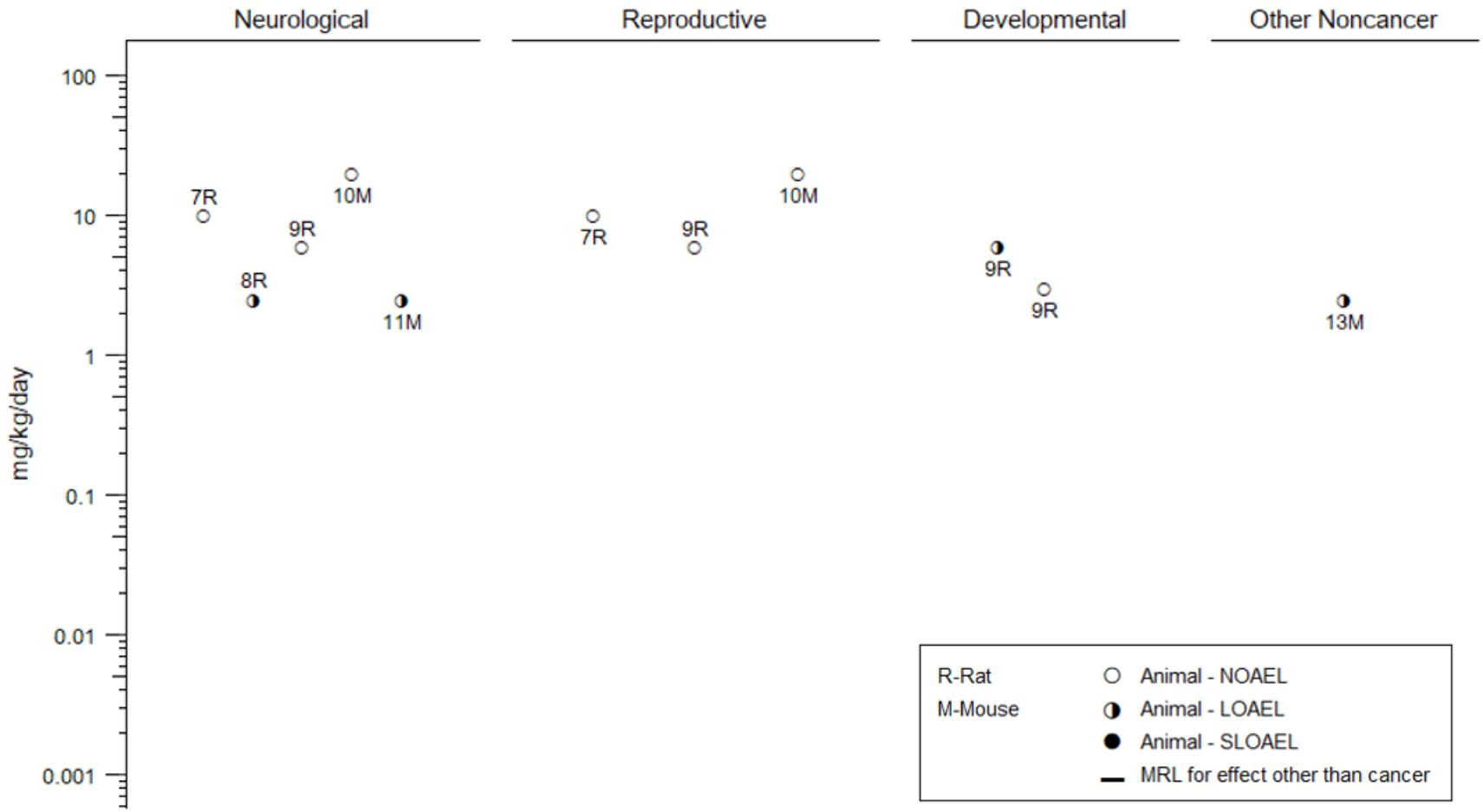

Figure 2-3. Levels of Significant Exposure to Acrolein – Oral
Chronic (≥365 days)

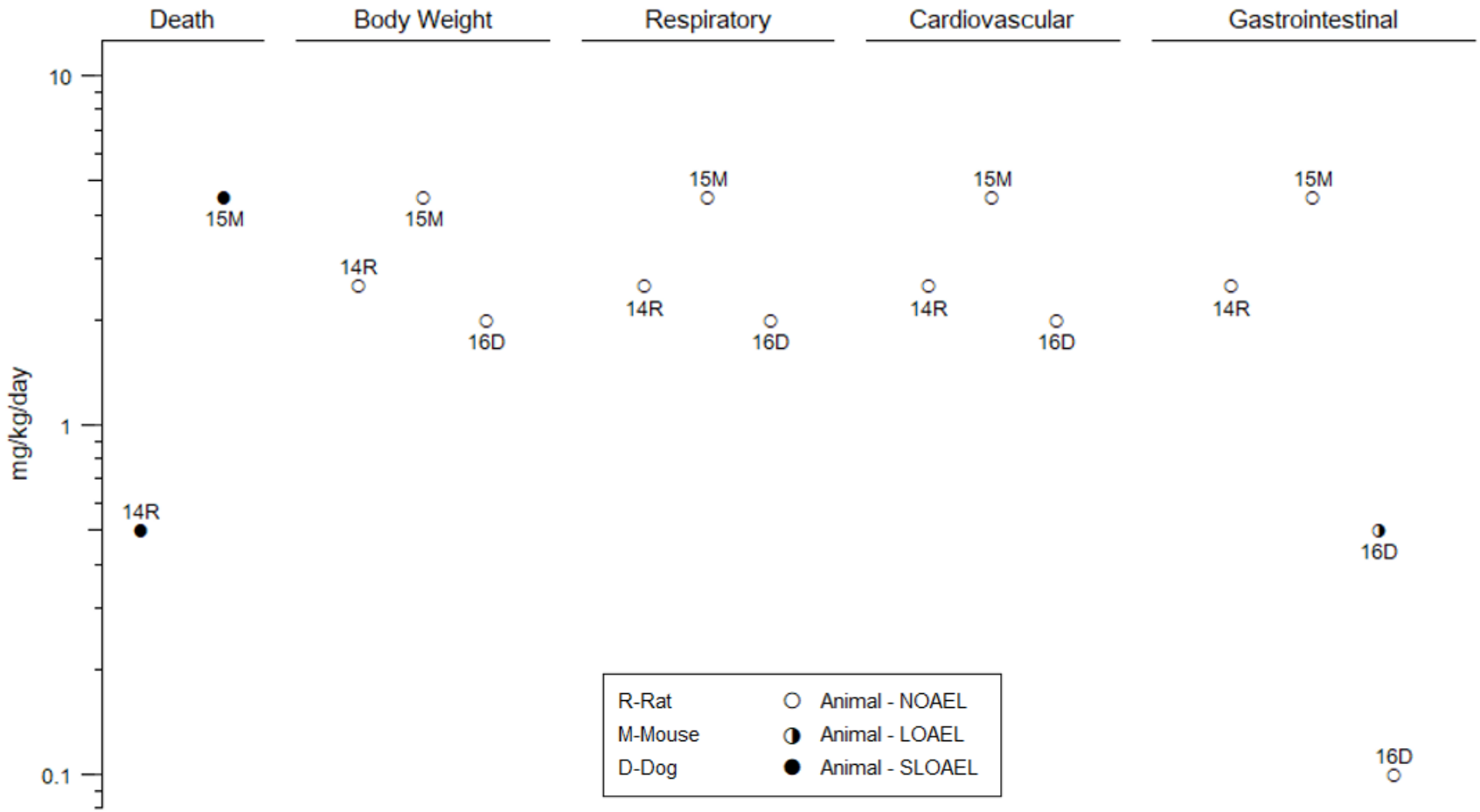

Figure 2-3. Levels of Significant Exposure to Acrolein – Oral
Chronic (≥365 days)

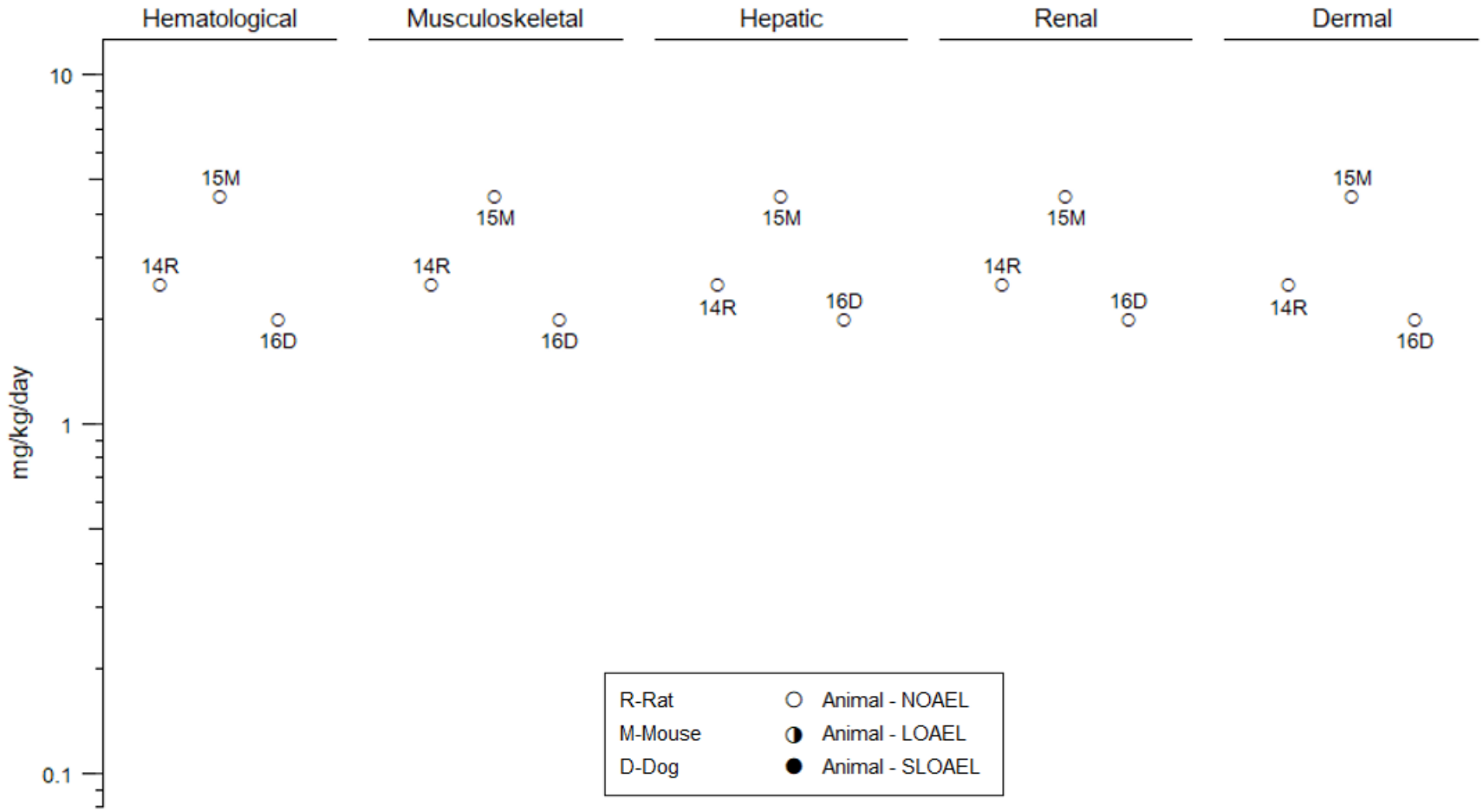

Figure 2-3. Levels of Significant Exposure to Acrolein – Oral
Chronic (≥365 days)

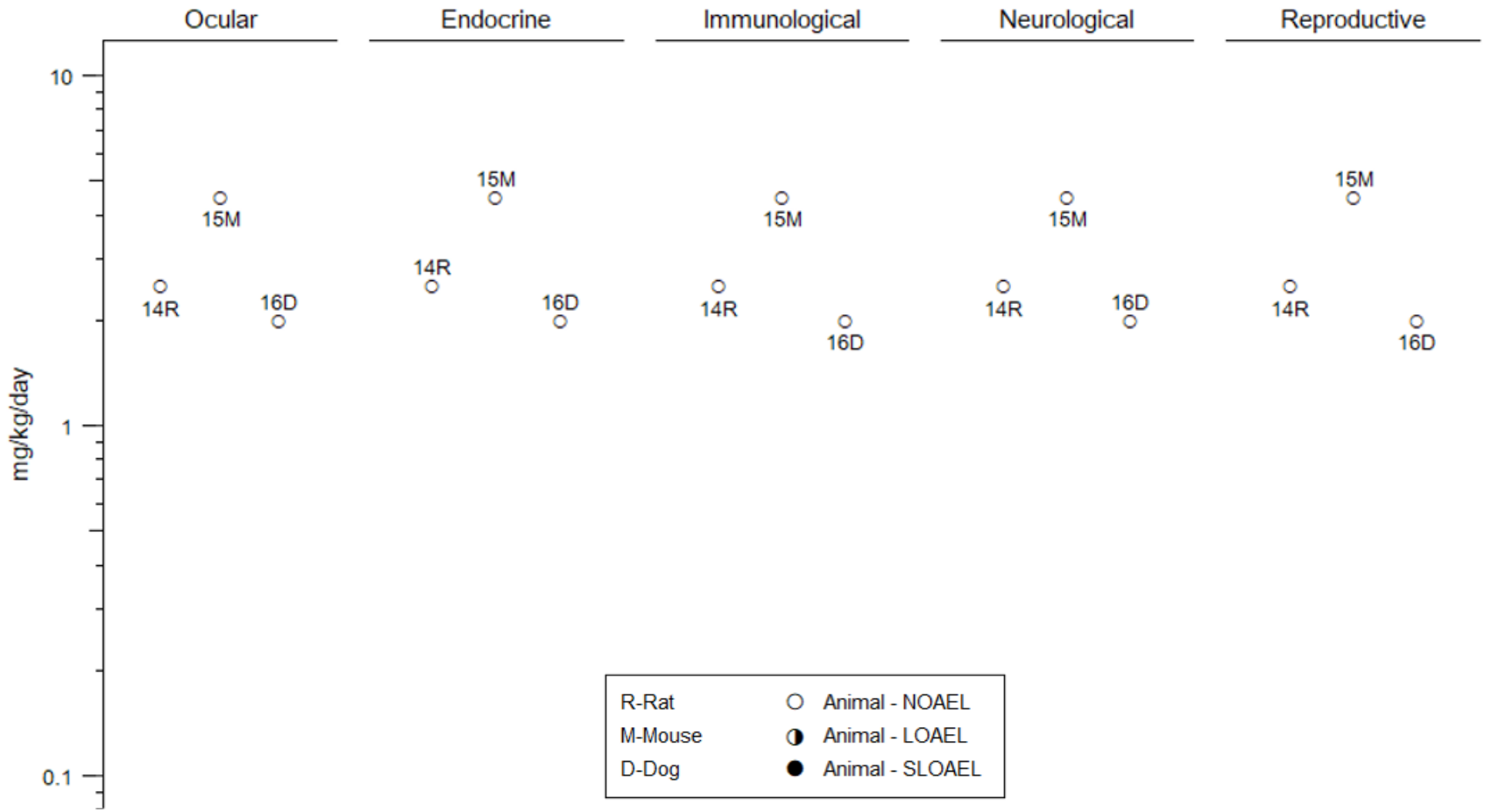

Table 2-3. Levels of Significant Exposure to Acrolein – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE								
Dwivedi et al. 2015								
Human 9 M, 9 F	2 hours	0, 0.05, 0.11 ppm	CS	Ocular	0.05	0.11		Eye irritation (blink rate and subjective symptoms)
Lacroix et al. 1976								
Human NS	Once	10%	CS, HP	Dermal			10	Severe skin irritation
Sim and Pattle 1957								
Human 24 M	5–10 minutes	0.81, 1.22 ppm	CS	Ocular		0.81		Eye irritation (lacrimation)
Weber-Tschopp et al. 1977								
Human 21 M, 25 F	1 hour	0, 0.3 ppm	CS	Ocular		0.3		Eye irritation (blink rate)
Ballantyne et al. 1989								
Rat (Sprague-Dawley) 5 M, 5 F	1 hour	14, 22, 24, 31, 81	LE	Ocular		14		Eye irritation (lacrimation)
Ballantyne et al. 1989								
Rat (Sprague-Dawley) 5 M, 5 F	4 hours	4.8, 7, 9.1, 12.1	LE	Ocular		4.8		Eye irritation (lacrimation)
Murphy et al. 1964								
Rat (Sprague-Dawley) 20 M	4 hours	0, 12 ppm	CS	Ocular			12	Severe eye irritation
Dachir et al. 2015								
Rabbit (New Zealand) 8–12 F	4 minutes	10, 20, 30 µL	CS, OP, HP	Ocular		10	30	LOAEL: Corneal erosions SLOAEL: Severe inflammation, corneal erosions, edema

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

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Gupta et al. 2020								
Rabbit (New Zealand) 6 B	1–5 minutes	0, 30 µL	OP, HP	Ocular			30	Severe eyelid swelling and inflammation, corneal opacity, excessive tear secretion, corneal edema
Skog 1950								
Rat (NS) 8 NS	30 minutes	44–305	CS, GN, HP	Ocular			44	Lacrimation
INTERMEDIATE EXPOSURE								
Lyon et al. 1970								
Monkey (Squirrel) 7–9 M	6 weeks 5 days/week 8 hours/day	0, 0.7, 3.7 ppm	CS	Ocular	0.7	3.7		Eye irritation (frequent blinking, eyes closed)
Lyon et al. 1970								
Monkey (Squirrel) 8–17 M	90 days 24 hours/day	0, 0.22, 1.0, 1.8 ppm	CS	Ocular	0.22	1		Eye irritation (eyes closed)
Lyon et al. 1970								
Rat (Sprague-Dawley) 7 M, 8 F	6 weeks 5 days/week 8 hours/day	0, 0.7, 3.7 ppm	CS	Ocular	3.7			
Lyon et al. 1970								
Rat (Sprague-Dawley) 7–15 M, 8–15 F	90 days 24 hours/day	0, 0.22, 1.0, 1.8 ppm	CS	Ocular	1.8			
Lyon et al. 1970								
Dog (Beagle) 2 M	6 weeks 5 days/week 8 hours/day	0, 0.7, 3.7 ppm	CS	Ocular	0.7	3.7		Eye irritation (blinking rate, eyes closed)
Lyon et al. 1970								
Dog (Beagle) 2–4 M	90 days 24 hours/day	0, 0.22, 1.0, 1.8 ppm	CS	Ocular	0.22	1		Eye irritation (ocular discharge)

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

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Feron et al. 1978								
Hamster (Golden Syrian) 10 M, 10 F	13 weeks 5 days/week 6 hours/day	0, 0.4, 1.4, 4.9 ppm	CS	Ocular	1.4	4.9		Eye irritation (eyes closed)
Lyon et al. 1970								
Guinea pig (Hartley) 7 M, 8 F	6 weeks 5 days/week 8 hours/day	0, 0.7, 3.7 ppm	CS	Ocular	3.7			
Lyon et al. 1970								
Guinea pig (Hartley) 6–15 M, 8–15 F	90 days 24 hours/day	0, 0.22, 1.0, 1.8 ppm	CS	Ocular	1.8			

B = both males and females; CS = clinical signs; F = female(s); GN = gross necropsy; HP = histopathology; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; OP = ophthalmology; SLOAEL = serious lowest-observed-adverse-effect level

2.2 DEATH

No studies were located regarding lethality in humans after exposure to acrolein from any route.

The data in experimental animals clearly indicate that respiratory toxicity is a primary cause of acrolein lethality following inhalation exposure and show an inverse relationship between the exposure concentration and the time it takes for death to occur after acute-duration exposures. Skog (1950) identified a 30-minute inhalation LC_{50} of 0.3 mg/L (130 ppm) in rats, while Ballantyne et al. (1989) reported 1- and 4-hour LC_{50} values of 26 and 8.3 ppm, respectively, in rats. A 30-minute inhalation LC_{50} of 225 ppm was reported for mice wherein wild-type males were more sensitive than females, but no age-related effects were observed (Conklin et al. 2017a). Increased mortality was seen in male mice exposed for 30 minutes to 75 ppm (100%) and 50 ppm (60%), and in female mice at 75 ppm (79%) within 5 days after treatment; no deaths were seen in females at 50 ppm (Bein et al. 2021).

Intermediate-duration inhalation studies have also reported 40–60% increases in mortality, particularly in rats exposed to acrolein concentrations ≥4 ppm (Costa et al. 1986; Feron et al. 1978; Kutzman et al. 1984, 1985; NTP 1981). Exposure to 4 ppm resulted in 100% mortality in a hypertension-sensitive rat strain, while a hypertension-resistant strain was somewhat protected (only 40% within 62 days of treatment) (Kutzman et al. 1984). No mortality was observed in rats exposed up to 1.733 ppm for 13 weeks (Dorman et al. 2008). Two out of 7 monkeys died following intermittent exposure to 3.7 ppm acrolein for 6 weeks (8 hours/day, 5 days/week), but no treatment-related deaths occurred in similarly treated dogs, guinea pigs, or rats, or in animals continuously exposed at lower concentrations for a longer period (≤1.8 ppm, 24 hours/day for 90 days) (Lyon et al. 1970). Weighted concentrations were calculated to compare the continuous (162 ppm-days) and intermittent (37 ppm-days) concentrations above and suggest that the monkeys were more sensitive. No exposure-related deaths occurred in rabbits and hamsters exposed to 4.9 ppm for 13 weeks (Feron et al. 1978) or in hamsters exposed to 4 ppm acrolein for 52 weeks (Feron and Kruysse 1977). In a 2-year chronic-duration inhalation study, only female rats had decreased survival at 2 ppm, while male rats and male and female mice had rates similar to controls (Matsumoto et al. 2021).

Increased mortality has also been reported following oral exposure to acrolein. Two oral LD_{50} values have been reported for acrolein: 10 mg/kg in female Wistar rats (Draminski et al. 1983) and 46 mg/kg in unspecified rats (Smyth et al. 1951). Additional acute-duration studies have observed >40% mortality with single gavage doses of 10 or 25 mg/kg in rats (Sakata et al. 1989; Sprince et al. 1979), although no

mortality was observed in mice given a single gavage dose of 5 mg/kg (Conklin et al. 2010). All Beagle dogs gavaged with 2.5 mg/kg/day (for 3 days), 5 mg/kg/day (for 2 days), or 10 mg/kg/day (once) were euthanized due to weight loss, excessive vomiting, or moribund state (Parent et al. 1992b). No mortality was observed in rats gavaged with 2.5 mg/kg/day for 8 weeks (Huang et al. 2013), or in mice gavaged with 1 mg/kg/day for 48 days (Ismahil et al. 2011). Parent et al. (1992b) (the range finding portion), Smyth et al. (1951), and Sakata et al. (1989) were not included in Table 2-2 or plotted in Figure 2-2 because of limited reporting or the absence of a control group.

Increased maternal mortality (30%) was observed in pregnant rats gavaged with 10 mg/kg/day on gestation days (GDs) 7–19 (EPA 1983), although no treatment-related deaths were observed in rabbits gavaged with 2 mg/kg/day on GDs 7–19 (Parent et al. 1993). In a set of 2-generation reproductive studies, increased mortality (20% in F0, 19% in F1) was observed in male and female rats gavaged with 6 mg/kg/day (Parent et al. 1992c).

Increased mortality (80–100% incidence) was also observed in rats (10 mg/kg/day) and mice (20 mg/kg/day) gavaged for 14 weeks (Auerbach et al. 2008; NTP 2006a), in male (but not female) mice (28% incidence) gavaged with 4.5 mg/kg/day for 18 months (Parent et al. 1991a), and in female (but not male) rats (incidence not reported) gavaged with 2.5 mg/kg/day for 106 weeks (Parent et al. 1992a). The overall survival rate was not affected in dogs exposed to 1.5–2 mg/kg/day by capsule dosing for 53 weeks (Parent et al. 1992b).

2.3 BODY WEIGHT

No studies were located regarding body weight changes in humans after inhalation, oral, or dermal exposure to acrolein.

Mixed results have been reported regarding body weight changes following inhalation exposure to acrolein. Decreased body weights (10–30%) have been observed following intermediate-duration exposure to concentrations as low as 0.55 ppm in rats (Bouley et al. 1975; Costa et al. 1986; Dorman et al. 2008; Feron et al. 1978; Kutzman et al. 1984; Leach et al. 1987; Lyon et al. 1970) and 4.9 ppm in rabbits and hamsters (Feron et al. 1978). In the Feron et al. (1978) study, decreased food consumption in rats and rabbits may have contributed to the observed body weight decrements. Exposure to acrolein for 2 years (5 days/week, 6 hours/day) resulted in decreased body weight in male rats (12% at 2 ppm) and male mice (17% at 1.6 ppm); no changes in body weights were seen in female rats or mice at these

concentrations (Matsumoto et al. 2021). Body weight was decreased by 10–11% in hamsters chronically exposed to 4 ppm acrolein for 52 weeks (Feron and Kruysse 1977). No differences in body weights were observed in guinea pigs, dogs, or monkeys intermittently exposed up to 3.7 ppm for 8 hours/day, 5 days/week for 6 weeks or exposed continuously up to 1.8 ppm for 90 days (Lyon et al. 1970). Similarly, no change in body weight was reported in hypertension-sensitive rats exposed to 1.4 ppm for 6 hours/day, 5 days/week for 62 days (Kutzman et al. 1984) or in rats exposed up to 1.07 ppm for 3 weeks (Leach et al. 1987). No changes in body weights were observed in mice exposed up to 1 ppm acrolein for 12 weeks (Conklin et al. 2017b).

No body weight changes were observed in mice gavaged with a single dose of up to 5 mg/kg (Conklin et al. 2010). Maternal extra-gestational body weight gain (final body weight-gravid uterus weight and initial body weight) was decreased 36% in pregnant rats gavaged with 6 mg/kg/day on GDs 7–19 compared to controls (EPA 1983). Rabbits gavaged with 2 mg/kg/day on GDs 7–19 exhibited reduced body weights early in the dosing schedule (GDs 7–10) but returned to weights similar to controls at the end of dosing (Parent et al. 1993). In a 2-generation reproductive study, no changes in body weights were observed in rats gavaged with up to 6 mg/kg/day (Parent et al. 1992c).

Decreased body weights were observed in male (22%) and female (10%) rats gavaged with 10 mg/kg/day for 14 weeks, but not in similarly exposed male and female mice (Auerbach et al. 2008; NTP 2006a). Mice gavaged with 2.5 mg/kg/day for 4 weeks showed a 15% decrease in body weight (Chen et al. 2019), although no difference in body weight was observed in mice gavaged with 1 mg/kg/day for 48 days (Ismahil et al. 2011) or in rats gavaged with 2.5 mg/kg/day for 8 weeks (Huang et al. 2013). No differences in body weights were observed in dogs given 2 mg/kg/day for 12 months (Parent et al. 1992b), in male and female mice gavaged with 4.5 mg/kg/day for 18 months (Parent et al. 1991a), or in rats gavaged with 2.5 mg/kg/day for 2 years (Parent et al. 1992a).

2.4 RESPIRATORY

Although human data are limited and often lack the necessary exposure information, the available studies point to the respiratory system as the primary target of inhaled acrolein. Several epidemiological studies have evaluated potential associations between acrolein exposure and reporting of respiratory symptoms, prevalence of asthma, and decrements in pulmonary function. Indoor acrolein concentrations were associated with self-reported sick building syndrome, including respiratory irritation symptoms (Sakellaris et al. 2021). Case reports of occupational workers exposed to acrolein have also reported

symptoms of respiratory toxicity. A case report details an aquatic pesticide worker who experienced throat tightness, difficulty breathing, inability to swallow, moderate phlegm production, and dyspnea following exposure to an unknown amount of acrolein, while two additional workers also experienced dyspnea (CDC 2013). In an occupational accident, an employee sprayed in the face and who breathed in high concentration of acrolein experienced dyspnea and chemical pneumopathy (Champeix et al. 1966). An association between indoor acrolein concentrations and increased prevalence of asthma was observed in a cross-sectional study of school children in France (Annesi-Maesano et al. 2012). Similar findings were reported in a general population study in the United States, where outdoor air concentrations of acrolein (0.05–0.46 $\mu g/m^3$) were associated with an increase in the prevalence of having at least one asthma attack in the prior year (deCastro et al. 2014). A case-control study of children with asthma in China, reported an increase in the probability of asthma associated with concentrations of a urinary metabolite of acrolein (3-hydroxypropylmercapturic acid [3-HPMA]) (Kuang et al. 2021). An increase in the concentration of urinary acrolein metabolites (3-HPMA and N-acetyl-S-(2-carboxyethyl)-L-cysteine [also known as carboxyethyl mercapturic acid or CEMA]) was also associated with decreased forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1) in an urban population in China (Wang et al. 2022). It should be noted that urinary 3-HPMA is not a specific biomarker for acrolein exposure and does not provide a means of distinguishing between exogenous and endogenous acrolein (see Section 3.3.1, Biomarkers of Exposure).

Human controlled exposure studies have also evaluated the respiratory effects of inhaled acrolein. Volunteers exposed to increasing levels of acrolein vapors for 40 minutes reported significant nose irritation at 0.26 ppm, throat irritation at 0.43 ppm, and a decrease in respiratory rate (25%) at 0.60 ppm (Weber-Tschopp et al. 1977). Severity of irritation was subjectively scored as “not at all” to “a little.” No significant difference was observed between controls and subjects exposed to 0.17 ppm. In the same study, nasal irritation was reported by subjects exposed to 0.6 ppm acrolein for 1.5 minutes, following prior exposure to lower concentrations (0.15, 0.30, and 0.45 ppm; 8-minute recovery between exposures). These experiments were not presented in Table 2-1 and Figure 2-2 due to changing exposure concentrations over time (continuous exposure) or exposure of the same subjects to multiple exposure concentrations (discrete exposure). The irritation response was reported to be stronger for continuous exposure compared to discrete exposure at the same concentration, suggesting that irritation severity increases over time with cumulative exposure (Weber-Tschopp et al. 1977). Constant exposure to 0.3 ppm acrolein for 60 minutes resulted in reports of mild nose irritation shortly after onset of exposure, while throat irritation was reported after 10 minutes (Weber-Tschopp et al. 1977). A significant decrease in respiratory rate (20%) occurred after 60 minutes of exposure to 0.3 ppm (Weber-Tschopp et al. 1977).

No change in pulmonary function (FVC, FEV1) or breathing frequency was observed in volunteers exposed to 0.11 ppm for 2 hours (Dwivedi et al. 2015).

The overall evidence from acute-, intermediate-, and chronic-duration inhalation studies in experimental animals indicates that the respiratory system is the primary target for acrolein. Acute-duration exposures for ≤1 hour at concentrations ≥0.3 ppm resulted in respiratory irritation, decreased pulmonary function, increased albumin in nasal lavage fluid (Morris 1996), increased lung weight, and respiratory tract histopathology in rats, mice, and guinea pigs (see Tables 2-4, 2-5, and 2-6). Changes in respiratory function persisted following removal of exposure (Conklin et al. 2017a). Several RD_{50} values (the concentration that suppresses the respiratory rate by 50%) are available for acrolein, ranging from 4.6 to 9.2 ppm in rats and from 1.03 to 2.9 ppm in mice (see Table 2-7). Acute-duration exposures for ≤1 hour at concentrations ≥250 ppm, resulted in labored breathing and lung edema (Conklin et al. 2017a; Skog 1950). Conklin et al. (2017a) found more effects in the upper airway as opposed to the lower airway and female rats showed less extreme nasal congestion and buildup of albumin in the lungs as evidenced by reduced conversion to mouth breathing compared to male rats following inhalation of high concentrations of acrolein.

Table 2-4. Respiratory Lesions in Animals Following Inhalation Exposure to Acrolein

Species; duration	Concentration (ppm)	Histology	Lesion details	Reference
Acute-duration				
Wistar rat; 3 days, 6 hours/day	0.25	↑	Nasal lesions (disarrangement and thickening of the respiratory epithelium, basal cell hyperplasia)	Cassee et al. 1996a
Fischer-344 rat; 14 days, 6 hours/day	0.586	↑	Nasal respiratory epithelial hyperplasia and epithelial squamous metaplasia in limited tissues	Dorman et al. 2008
Wistar rat; 6 hours	1.4	↔	Nasal cavity	Cassee et al. 1996a
Swiss Webster mouse; 5 days, 6 hours/day	1.7	↑ ↔	Nasal lesions (ulceration, necrosis, and squamous metaplasia of respiratory and olfactory epithelium) Trachea, lungs	Buckley et al. 1984

Table 2-4. Respiratory Lesions in Animals Following Inhalation Exposure to Acrolein

Species; duration	Concentration (ppm)	Histology	Lesion details	Reference
Fischer-344 rat; 14 days, 6 hours/day	1.8	↑	Olfactory epithelial atrophy (dorsal meatus, septum, ethmoid turbinate)	Dorman et al. 2008
Wistar rat; 4 hours	2	↑	Lung lesions (epithelial cell sloughing and mononuclear cells in the bronchioles, hyperemia, emphysema)	Arumugam et al. 1999a
C57BL/6J mouse; 3 days, 6 hours/day	5	↔	Lungs	Kasahara et al. 2008
Hotzman rat; 4 hours	8	↑	Pulmonary edema, inflammation	Murphy 1965
C57BL/6J mouse; 12 hours	10	↑	Lung lesions (air space enlargement)	Kim et al. 2020
129X1/SvJ mouse; 6–17 hours	10	↑	Lung lesions (perivascular enlargement, leukocyte infiltration)	Leikauf et al. 2011
SM/J mouse; 6–17 hours	10	↑	Lung lesions (perivascular enlargement, leukocyte infiltration)	Leikauf et al. 2011
Rat (NS); 30 minutes	44–305	↑	Lung lesions (edema, hyperemia, and hemorrhages, degenerative changes in the bronchial epithelium)	Skog 1950
B6C3F1 mouse; 30 minutes	50 M 75 F	↑	Lung lesions (alveolar wall thickening, proteinaceous deposit, leukocyte infiltrates)	Bein et al. 2021
C57BL/6J mouse; 30 minutes	250	↑	Nasal and tracheal lesions (epithelial sloughing, mucus accumulation, inflammatory cell infiltration)	Conklin et al. 2017a
Intermediate-duration				
Beagle dog; 90 days continuously	0.22	↑	Emphysema, acute congestion, focal vacuolization of the bronchiolar epithelial cells, constriction of the bronchioles	Lyon et al. 1970

Table 2-4. Respiratory Lesions in Animals Following Inhalation Exposure to Acrolein

Species; duration	Concentration (ppm)	Histology	Lesion details	Reference
Fischer-344 rat; 13 weeks, 5 days/week, 6 hours/day	0.586	↑ ↑	Nasal lesions (respiratory epithelial hyperplasia [dorsal meatus and lateral wall] and epithelial squamous metaplasia [lateral wall]), most resolving with post-exposure recovery period Laryngeal lesions (respiratory epithelial squamous metaplasia)	Dorman et al. 2008
Squirrel monkey; 6 weeks, 5 days/week, 8 hours/day	0.7	↑	Chronic inflammation of the lung	Lyon et al. 1970
Sprague-Dawley rat; 6 weeks, 5 days/week, 8 hours/day	0.7	↑	Chronic inflammation of the lung	Lyon et al. 1970
Beagle dog; 6 weeks, 5 days/week, 8 hours/day	0.7	↑	Chronic inflammation of the lung	Lyon et al. 1970
Hartley guinea pig; 6 weeks, 5 days/week, 8 hours/day	0.7	↑	Chronic inflammation of the lung	Lyon et al. 1970
Sprague-Dawley rat; 90 days continuously	1.0	↑	Pulmonary hemorrhage	Lyon et al. 1970
Hartley guinea pig; 90 days continuously	1.0	↑	Pulmonary inflammation	Lyon et al. 1970
Wistar rat; 13 weeks, 5 days/week, 6 hours/day	1.4	↑ ↔	Nasal lesions (squamous metaplasia, neutrophilic infiltration) Lungs	Feron et al. 1978
Syrian hamster; 13 weeks, 5 days/week, 6 hours/day	1.4	↑ ↔	Nasal lesions (inflammation) Lungs	Feron et al. 1978

Table 2-4. Respiratory Lesions in Animals Following Inhalation Exposure to Acrolein

Species; duration	Concentration (ppm)	Histology	Lesion details	Reference
Fischer-344 rat; 13 weeks, 5 days/week, 6 hours/day	1.8	↑ ↑	Nasal lesions (respiratory epithelial hyperplasia; respiratory and olfactory epithelial squamous metaplasia) throughout the nose Lesions in the larynx (epithelial inflammation; olfactory epithelial squamous metaplasia) and trachea (olfactory epithelial squamous metaplasia)	Dorman et al. 2008
Squirrel monkey; 90 days, continuously	1.8	↑	Tracheal squamous metaplasia and basal cell hyperplasia	Lyon et al. 1970
Sprague-Dawley rat; 3 weeks, 5 days/week, 6 hours/day	3.0	↑ ↔	Nasal lesions (squamous metaplasia and degeneration of the respiratory epithelium, neutrophil infiltration, degeneration and atrophy of the olfactory epithelium) Lungs	Leach et al. 1987
Sprague-Dawley rat; 4 weeks, 5 days/week, 5 hours/day	3.1	↑	Laryngeal lesions (epithelial sloughing, cell death, edema)	Liu et al. 2019
Squirrel monkey; 6 weeks, 5 days/week, 8 hours/day	3.7	↑	Hemorrhagic spots in lungs	Lyon et al. 1970
Fischer-344 rat; 62 days, 5 days/week, 6 hours/day	4.0	↑	Bronchiolar epithelial necrosis, bronchiolar edema fluid, acute rhinitis, tracheal edema	Costa et al. 1986; Kutzman et al. 1985; NTP 1981
Dutch rabbit; 13 weeks, 5 days/week, 6 hours/day	4.9	↑ ↑ ↑	Nasal lesions (necrotizing rhinitis, neutrophilic infiltration) Lung lesions (bronchitis, hyperplasia, metaplasia) Tracheal lesions (hyperplastic epithelium, mucus cells)	Feron et al. 1978
Chronic-duration				
B6D2F1/Crlj mouse; 2 years, 5 days/week, 6 hours/day	0.4	↑ F	Nasal lesions (inflammation, hyperplasia, metaplasia, regeneration)	Matsumoto et al. 2021

Table 2-4. Respiratory Lesions in Animals Following Inhalation Exposure to Acrolein

Species; duration	Concentration (ppm)	Histology	Lesion details	Reference
Fischer-344/DuCrlCrlj rat; 2 years, 5 days/week, 6 hours/day	2.0	↑	Nasal lesions (inflammation, metaplasia, eosinophilic changes, goblet cell hyperplasia)	Matsumoto et al. 2021
B6D2F1/Crlj mouse; 2 years, 5 days/week, 6 hours/day	1.6	↑ M	Nasal lesions (inflammation, hyperplasia, metaplasia, regeneration)	Matsumoto et al. 2021
Syrian hamster; 52 weeks, 5 days/week, 7 hours/day	4	↑ ↔	Nasal lesions (inflammation and epithelial metaplasia, neutrophilic infiltrates, submucosa thickening) Lungs	Feron and Kruysse 1977

↑ = increase in histopathological lesions; ↔ = no change; F = female(s); F-344 = Fischer-344; M = male(s); NS = not specified

Table 2-5. Respiratory Function in Animals Following Inhalation Exposure to Acrolein

Species; duration	Concentration (ppm)	Effect	Respiratory function	Reference
Acute-duration				
Guinea pig (NS); 2 hours	0.6	↑ ↓	Resistance, tidal volume Respiration rate	Murphy et al. 1963
C57BL/6N mouse; 10 minutes	1.3	↑ ↓	Resistance Respiration rate	Morris et al. 2003
Spontaneous hypertensive rat; 3 hours	2.9	↑	Decreased breathing frequency, increased expiratory time	Perez et al. 2015
Sprague-Dawley rat; 3 hours	3.0	↑ ↓	Respiratory irritation (upper and lower airways) Respiration rate	Hazari et al. 2008
Spontaneous hypertensive rat; 3 hours	3	↑	Increased breathing frequency and minute volume	Perez et al. 2013

Table 2-5. Respiratory Function in Animals Following Inhalation Exposure to Acrolein

Species; duration	Concentration (ppm)	Effect	Respiratory function	Reference
WKY rat; 3 hours	3	↔		Perez et al. 2013
C57BL/6 mouse; 6 hours	3	↑	Expiratory time, tidal volume	Kurhanewicz et al. 2017
		↓	Respiration rate	
Wistar rat; 1–2 days, 4 hours/day	4	↑	Inspiratory and expiratory time, labored breathing	Snow et al. 2017
		↔	Breathing frequency, minute volume, tidal volume	
Sprague-Dawley rat; 4 hours	4.8	↓	Decreased breathing rate and a conversion to mouth breathing	Ballantyne et al. 1989
Sprague-Dawley rat; 60 minutes	14	↓	Decreased breathing rate and a conversion to mouth breathing	Ballantyne et al. 1989
Guinea pig (NS); 60 minutes	17	↑	Resistance, tidal volume	Davis et al. 1967
		↓	Respiration rate, minute volume	
C57BL/6J mouse; 30 minutes	250	↑	Expiratory and inspiratory time	Conklin et al. 2017a
		↓	Respiration rate	
Intermediate-duration				
Fischer-344 rat; 62 days, 5 days/week, 6 hours/day	4	↑	Resistance, tidal volume	Costa et al. 1986; Kutzman et al. 1985; NTP 1981
		↓	Respiration rate	

↑ = increase; ↓ = decrease; ↔ = no change; NS = not specified; WHY = Wistar Kyoto

Table 2-6. Lung Weight in Animals Following Inhalation Exposure to Acrolein

Species; duration	Concentration (ppm)	Effect	Percent change	Reference
Acute-duration				
Hotzman rats; 4 hours	8	↑	19% (relative)	Murphy 1965
C57BL/6J mouse; 30 minutes	250	↑	75% (males)	Conklin et al. 2017a

Table 2-6. Lung Weight in Animals Following Inhalation Exposure to Acrolein

Species; duration	Concentration (ppm)	Effect	Percent change	Reference
Intermediate-duration				
C57BL/6J mouse; 12 weeks, 5 days/week, 6 hours/day	1	↔		Conklin et al. 2017b
Wistar rat; 13 weeks, 5 days/week, 6 hours/day	1.4	↑ ↑	13% (males) 26% (females)	Feron et al. 1978
Syrian hamster; 13 weeks, 5 days/week, 6 hours/day	1.4	↑ ↑	34% (males) 18% (females)	Feron et al. 1978
Fischer-344 rat; 62 days, 5 days/week, 6 hours/day	4	↑	66%	Costa et al. 1986; Kutzman et al. 1985; NTP 1981
Dutch rabbit; 13 weeks, 5 days/week, 6 hours/day	4.9	↔		Feron et al. 1978

↑ = increase in lung weight; ↔ = no change in lung weight; NS = not specified; OFA = Oncins France Strain A

Table 2-7. RD_{50} Values in Animals Following Inhalation Exposure to Acrolein

Species; duration	Concentration (ppm)	RD_{50} (ppm)	Reference
Acute-duration			
B6C3F1 mouse; 10 minutes	0.04–8	1.41	Steinhagen and Barrow 1984
Swiss Webster mouse; 10 minutes	0.04–8	1.03	Steinhagen and Barrow 1984
C57BL/6J mouse; 10 minutes	0.3–3.9	1.59	Morris et al. 2003
Swiss Webster mouse; 10 minutes	0–10	1.7	Kane and Alarie 1977
CF-1 mouse; 30 minutes	0.85–7.25	2.9	Nielsen et al. 1984
Wistar rat; 20 minutes	6.7–54	4.6	Bergers et al. 1996
Fischer-344 rats; 10 minutes	0.5–10	6	Babiuk et al. 1985
Wistar rats; 30 minutes	1.7–32	9.2	Cassee et al. 1996b

RD_{50} = exposure concentration producing a 50% respiratory rate decrease

Similar effects have been observed at the highest concentrations following acute-duration acrolein exposures ≥1 hour, although the effects are often more severe. Decreased respiratory function, increased

lung weights, and nasal and pulmonary lesions and inflammation are commonly reported in rodent studies with the most sensitive portion being the lateral wall at level II of the nasal cavity (see Tables 2-4, 2-5, and 2-6). Exposure to 2.9 ppm acrolein for 3 hours altered the breathing frequency, minute volume, and expiratory time in a spontaneous hypertensive rat model, but not in the similarly exposed normotensive rat line (Perez et al. 2013, 2015), suggesting a relationship between the observed respiratory and cardiovascular effects. Similar effects were observed in another study, where the incidence of mild, terminal bronchiolar hyperplasia occurred at a higher incidence in hypertension-sensitive rats compared to hypertension-resistant rats; however, the concentration at which these effects occurred was not clearly reported (Kutzman et al. 1984).

Nasal lesions including ulceration, necrosis, inflammation, and squamous hyperplasia and metaplasia of the respiratory and olfactory epithelium appear to be the most common effects of acrolein exposure following acute-, intermediate-, and chronic-duration inhalation in rodents (see Table 2-4). Additional changes in lung weights and tracheal and pulmonary histopathology have also been observed in longer-duration studies (see Tables 2-5 and 2-6). Alveolar edema was noted only in rats that died from 13-week acrolein exposure (Feron et al. 1978).

As expected, oral studies in animals do not produce the same respiratory effects observed following inhalation exposure. No histopathological changes were reported in the lungs of rats exposed to a single dose of 25 mg/kg acrolein (Sakata et al. 1989), although wheezing was observed in pregnant rats gavaged with ≥3.6 mg/kg/day for 13 days (EPA 1983). Sakata et al. (1989) was not included in Table 2-2 or plotted in Figure 2-3 because a control group was not included. Abnormal breathing (wheezing, dyspnea) was also a common observance in rats and mice following intermediate-duration gavage exposure to concentrations ≥5 mg/kg/day (Auerbach et al. 2008; NTP 2006a; Parent et al. 1992c). Additional histopathological analyses reveal lung congestion in rats gavaged with 6 mg/kg/day (Parent et al. 1992c), and acute nasal inflammation in rats gavaged with 10 mg/kg/day (Auerbach et al. 2008; NTP 2006a). In contrast, histopathological examination of the respiratory system (i.e., lungs, trachea) revealed no effects after intermediate-duration oral exposure to acrolein in rats or mice (Auerbach et al. 2008; NTP 2006a) or chronic-duration exposure in rats (Parent et al. 1992a), mice (Parent et al. 1991a), or dogs (Parent et al. 1992b).

Immunological effects of acrolein in the respiratory tract, including altered responses to allergen or bacterial challenge, are discussed in the Section 2.14 (Immunological).

Mechanisms. The molecular mechanisms of acrolein toxicity are discussed in detail in Section 2.21, Mechanisms of Toxicity. Yeager et al. (2016) proposed a mode of action for acrolein-induced respiratory effects, with a focus on respiratory effects associated with tobacco smoking (e.g., chronic obstructive pulmonary disorder [COPD]). The key events in this mode-of-action analysis, which are consistent with the mechanisms outlined in Section 2.21, are as follows: (1) direct interaction with cellular proteins and macromolecules; (2) increased oxidative stress, oxidative damage, and inflammation; and (3) cell death via apoptosis, necrosis, and oncosis (cell death by swelling); and tissue destruction and remodeling.

2.5 CARDIOVASCULAR

Human studies examining cardiovascular effects are limited, primarily due to lack of exposure information. Urinary levels of the acrolein metabolite, 3-HPMA, were associated with increased risk of cardiovascular disease (DeJarnett et al. 2014) and higher blood pressure (McGraw et al. 2021) in participants of the Louisville Healthy Heart Study. Plasma levels of 3-HPMA were also associated with cardiovascular disease diagnoses, independent of smoking status (Lorenz et al. 2021). A population-based study identified an association between urinary acrolein metabolites and dyslipidemia risk (Feng et al. 2022a).

Several acute-duration inhalation studies have identified cardiovascular effects in rodents. Exposure to 44–305 ppm acrolein for 30 minutes resulted in hyperemia of the heart in rats (Skog 1950). Blood oxygen saturation and cardiac output were decreased in male (but not female) mice exposed to 250 ppm acrolein for 30 minutes (Conklin et al. 2017a). Mice exposed to 3 ppm acrolein for 3 hours showed increased heart rate variability and an increase in the number of arrhythmias (Kurhanewicz et al. 2017, 2018), while rats experienced decreased heart rates (Hazari et al. 2008).

Following intermediate-duration exposure to acrolein, increased relative heart weights have been observed primarily in male rats (22%) and female hamsters (11%) exposed to concentrations as low as 4 ppm, but body weights were decreased at the same exposure concentrations (Feron et al. 1978; NTP 1981). In contrast, other studies in hamsters, rabbits, and mice have not identified changes in cardiovascular organ weights (Conklin et al. 2017b; Feron and Kruysse 1977; Feron et al. 1978), and no associated histopathology was found in any species. Nonspecific inflammatory changes in the heart were reported in rats, dogs, monkeys, and guinea pigs after a continuous 90-day exposure to 0.22 ppm acrolein (Lyon et al. 1970), although the toxicological significance of these changes is unknown. No alterations in

heart rate (NTP 1981) or blood pressure (Kutzman et al. 1984) were observed in rats exposed to 4 ppm acrolein for 62 days.

Mice gavaged with 1 mg/kg/day acrolein for 48 days showed signs of cardiomyopathy, including myocardial inflammation, myocyte hypertrophy and cell death, and left ventricle remodeling and dysfunction (Ismahil et al. 2011). In contrast, histopathological examination of the cardiovascular system revealed no effects after longer, intermediate-duration oral exposure to higher doses of acrolein in rats (up to 10 mg/kg/day) or mice (up to 20 mg/kg/day) (NTP 2006a; Parent et al. 1992c) or chronic-duration exposure in rats (up to 2.5 mg/kg/day), mice (up to 4.5 mg/kg/day), or dogs (up to 2 mg/kg/day) (Parent et al. 1991a, 1992a, 1992b). In addition, no changes in blood pressure were observed in rats gavaged with 2.5 mg/kg/day for 8 weeks (Huang et al. 2013). Absolute heart weight was decreased in female rats gavaged for 14 weeks with ≥5 mg/kg/day (5 days/week); however, no histological changes were seen (NTP 2006a).

Several studies have examined the cardiovascular effects of acrolein exposure in rodent models of disease, including genetic knockout animals. Exposure to 2.9 ppm acrolein for 3 hours caused decreased arterial blood oxygen and increased arterial blood carbon dioxide and heart rate in a spontaneous hypertensive rat model, but not in the similarly exposed normotensive rat line, although blood pressure was increased in both strains. (Perez et al. 2013, 2015). Other studies have looked at the effects of acrolein in a cardiovascular disease mouse model prone to atherosclerosis, apoE$^{-/-}$ mice. Gavage exposure to 2.5 mg/kg/day acrolein for 8 weeks resulted in an increase in lesions and macrophage accumulation in the aorta (Srivastava et al. 2011), while oral exposure to 3 mg/kg/day in drinking water for 1 month caused an increase in aortic cholesterol, triglycerides, and lipid peroxides (Rom et al. 2017). A knockout mouse model for an ion channel protein TRPA1 showed resistance to the cardiovascular effects observed in wild-type mice following acrolein exposure (Kurhanewicz et al. 2017, 2018).

2.6 GASTROINTESTINAL

No studies were located regarding gastrointestinal effects in humans following exposure to acrolein by any route.

Gastrointestinal effects following inhalation exposure are limited. Exposure to high concentrations (100–275 ppm) of acrolein by inhalation for 10–30 minutes caused rats, which are obligate nose breathers, to convert to mouth breathing resulting in notable air ingestion and thus proximal gastrointestinal tract

distension (morphometrically) (Conklin et al. 2017a). Other gastrointestinal tract parameters were not reported.

Gastrointestinal irritation is the primary effect of oral exposure to acrolein; no studies were located regarding gastrointestinal effects in animals following dermal exposure to acrolein. Rats administered a single gavage dose of 25 mg/kg of acrolein showed severe multifocal ulceration of the forestomach and glandular stomach 48 hours after dosing, although no controls were used for comparison. The areas of ulceration showed severe inflammation, focal hemorrhage, and edema (Sakata et al. 1989). Due to the lack of a control group, this study was not included in Table 2-2 or plotted in Figure 2-3.

Similar lesions have been observed following intermediate-duration exposure. Stomach lesions, including ulcers, hemorrhage, hyperplasia of the forestomach, and erosion of the glandular mucosa, were found in 2 generations of rats gavaged with 6 mg/kg/day (Parent et al. 1992c). Forestomach squamous epithelial hyperplasia was observed in male and female rats gavaged with ≥5 and ≥2.5 mg/kg/day, respectively, and in mice gavaged with ≥2.5 mg/kg/day for 14 weeks (Auerbach et al. 2008; NTP 2006a). At higher concentrations, glandular stomach hemorrhage was observed in rats gavaged with 10 mg/kg/day and in mice gavaged with 20 mg/kg/day, while stomach necrosis was also observed in mice (Auerbach et al. 2008; NTP 2006a).

Although the reported lesions are consistent and dose-related for intermediate-duration exposures, possible adaptation to irritating effects may have important implications for chronic-duration exposures. No significant gastrointestinal effects (i.e., histopathology) of acrolein exposure were reported in rats (Parent et al. 1992a) or mice (Parent et al. 1991a) after chronic-duration gavage dosing with up to 2.5 or 4.5 mg/kg/day, respectively. While no unusual gross or significant histological lesion in the gastrointestinal tract were observed in dogs given up to 2 mg/kg/day for 53 weeks (Parent et al. 1992b), increased incidences of vomiting were observed during and shortly after dosing (beginning at 0.5 mg/kg/day), suggesting gastrointestinal irritation. However, adaptation seemed to occur, as vomiting frequency near the end of the study was reduced compared to the first 4 weeks of the study in high-dose animals.

2.7 HEMATOLOGICAL

No studies were located regarding hematological effects in humans following exposure to acrolein by any route.

Hematological changes, particularly alterations in white blood cell counts, have been observed in rodents following inhalation exposure to acrolein. Lymphocytes were increased and neutrophils were decreased in male mice (but not female mice) exposed to 250 ppm acrolein for 30 minutes (Conklin et al. 2017a), although rats exposed to 4 ppm over 1 or 2 days had no differences in total white blood cells or lymphocytes (Snow et al. 2017). Platelet and platelet-leukocyte aggregation, and increased platelet-fibrinogen binding were observed in mice exposed by inhalation to 4.9 ppm acrolein for 6 hours or 1.1 ppm for 6 hours/day for 4 days (Sithu et al. 2010). Calculations for time-weighted concentrations (ppm-hour) for comparison between single (29.4 ppm-hour) and intermittent (26.4 ppm-hour) dosing suggest similar cumulative exposures.

Female hamsters exposed to 4.9 ppm acrolein for 13 weeks had increased numbers of erythrocytes, packed cell volume, hemoglobin content, and lymphocyte count, and decreased numbers of neutrophilic leukocytes, although these differences were not observed in male hamsters, rats, or rabbits (Feron et al. 1978). Similarly, female hamsters exposed to 4 ppm acrolein for 52 weeks had increased hemoglobin content and packed cell volume, although this was not observed in similarly exposed males, and no other alterations in hematological parameters were reported (Feron and Kruysse 1977). No adverse hematological effects were observed following intermediate-duration exposure in rats (NTP 1981), guinea pigs, dogs, or monkeys (Lyon et al. 1970), or following chronic-duration exposure in rats or mice (Matsumoto et al. 2021).

A single oral dose of 5 mg/kg in mice increased ADP-induced platelet and platelet-leukocyte aggregations and reduced the bleeding time (Sithu et al. 2010). Longer duration oral exposure does not result in the same hematological effects that are seen with inhalation exposure. Increased platelet and reticulocyte counts were observed in rats gavaged with 5 mg/kg/day for 14 weeks (Auerbach et al. 2008; NTP 2006a), while bone marrow hyperplasia was observed at 10 mg/kg/day. Decreased serum albumin, calcium, and total protein levels, and changes in red blood cell parameters were seen in Beagle dogs gavaged with 2 mg/kg/day for 12 months (Parent et al. 1992b); however, the toxicological significance of this is unclear. No pathological changes in liver or kidney were observed that would support these changes. Furthermore, extensive vomiting was seen in these animals and could have contributed to the changes. No altered hematological effects were observed in mice given a single gavage dose of up to 5 mg/kg/day (Conklin et al. 2010), gavage doses up to 10 mg/kg/day, 5 days/week for 14 weeks (Auerbach et al. 2008; NTP 2006a), or gavage doses of up to 4.5 mg/kg/day acrolein for 18 months (Parent et al. 1991a); in rats

given gavage doses of up to 2.5 mg/kg/day for 2 years (Parent et al. 1992a); or in dogs given 2 mg/kg/day for 12 months (Parent et al. 1992b).

2.8 MUSCULOSKELETAL

No studies were located regarding musculoskeletal effects in humans following inhalation, oral, or dermal exposure to acrolein.

Few studies have examined potential musculoskeletal effects in animals following acrolein exposure. Plasma creatine kinase levels were unchanged in mice exposed to 4.9 ppm acrolein for 6 hours, or to 1.1 ppm for 6 hours/day for 4 days (Sithu et al. 2010). Similar results were observed in mice exposed to 1 ppm for 12 weeks (Conklin et al. 2017b).

The weight and cross-sectional area of the soleus muscle were decreased in mice gavaged with 2.5 mg/kg/day acrolein for 4 weeks (Chen et al. 2019). No histopathological changes were observed in musculoskeletal tissues after intermediate-duration oral exposure in rats or mice (Auerbach et al. 2008; NTP 2006a) or in chronically exposed rats (Parent et al. 1992a), mice (Parent et al. 1991a), or dogs (Parent et al. 1992b).

2.9 HEPATIC

Studies examining the potential hepatic effects of acrolein exposure in humans are limited. The urinary acrolein metabolites, CEMA and 3-HPMA, were associated with elevated levels of alkaline phosphatase (ALP) in subjects from the Health, Environment, and Action in Louisville (HEAL) study, while the metabolite, 3-HPMA, was positively associated with bilirubin in nonsmokers in the study (Wahlang et al. 2022).

Mixed results have been reported for hepatic effects in experimental animal studies. Single 30-minute inhalation exposures between 44 and 305 ppm have resulted in hyperemia, perivascular edema, and necroses of the liver in rats (Skog 1950) and increased serum triglycerides in mice (Conklin et al. 2017a). In rats following 4-hour exposures to 4–8 ppm acrolein for 1 or 2 days, alterations in ALP activity (Murphy 1965; Murphy et al. 1964), cholesterol levels (Snow et al. 2017), and liver weights (Murphy et al. 1964) have been observed. Decreases in serum cholesterol and triglyceride levels were seen in mice exposed to 1.1 ppm of acrolein 6 hours/day for 4 days. No significant changes in these parameters were

reported in mice exposed to 5 ppm acrolein for 6 hours in a single-day exposure, which resulted in a similar cumulative exposure (Sithu et al. 2010). The biological relevance of the lipid decreases is unclear considering that other studies reported significant increases in these parameters following inhalation and oral exposures to acrolein (Conklin et al. 2010; Rom et al. 2017; Snow et al. 2017). Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were unchanged in mice exposed to 4.9 ppm acrolein for 6 hours or 1.1 ppm of acrolein 6 hours/day for 4 days (Sithu et al. 2010).

Continuous exposure to ≥1 ppm for 90 days led to focal liver necrosis in rats and guinea pigs (Lyon et al. 1970); however, other intermediate-duration studies that employed intermittent exposures to 1.4–4.9 ppm acrolein for 6–8 hours/day, 5 days/week for 6–13 weeks did not result in histopathological changes in the liver of monkeys, rats, guinea pigs, hamsters, rabbits, or dogs (Feron et al. 1978; Kutzman et al. 1985; Lyon et al. 1970; NTP 1981). Relative liver weights, ALP, ALT, and AST were increased in hypertension-resistant rats exposed to 4 ppm acrolein for 62 days (Kutzman et al. 1984), but not in hypertension-sensitive rats (Kutzman et al. 1984) or F-344 rats (Kutzman et al. 1985; NTP 1981). No changes in ALP, ALT, or AST were seen in monkeys, rats, guinea pigs, or dogs following exposure of up to 3.7 ppm for 8 hours/day, 5 days/week for 6 weeks (Lyon et al. 1970) or in mice exposed up to 1 ppm for 6 hours/day, 5 days/week for 12 weeks (Conklin et al. 2017b). In chronic-duration studies, female hamsters showed a decrease in absolute liver weights following exposure to 4 ppm acrolein for 52 weeks, but there were no differences in male liver weights and no associated histopathology (Feron and Kruysse 1977). No exposure-related changes in liver weights, histopathology, or clinical chemistry were observed in rats or mice chronically exposed to 2 or 1.6 ppm acrolein, respectively (Matsumoto et al. 2021).

A single gavage dose of 5 mg/kg resulted in increased plasma cholesterol, phospholipids, and triglycerides in mice (Conklin et al. 2010), while 25 mg/kg of acrolein resulted in eosinophilic degeneration of the liver in rats 48 hours after dosing (Sakata et al. 1989). Sakata et al. (1989) was not included in Table 2-2 or plotted in Figure 2-3 because a control group was not included. In an intermediate-duration study increased liver weights were observed in female rats (≥ 5 mg/kg/day) and male mice (10 mg/kg/day) (Auerbach et al. 2008; NTP 2006a). Male and female rats also exhibited increased ALP activity and decreased serum albumin at doses ≥2.5 mg/kg/day. However, since no histopathological changes were observed in the livers of either species (Auerbach et al. 2008; NTP 2006a), the toxicological significance is unknown and these changes may reflect adaptive responses. No liver effects were observed upon gross pathological or histological examinations in rats after intermediate-duration exposure up to 7.2 mg/kg/day acrolein (Parent et al. 1992c). Similarly, no

significant liver histopathology was observed in chronically exposed rats (Parent et al. 1992a), mice (Parent et al. 1991a), or dogs (Parent et al. 1992b) at doses of 2–4.5 mg/kg/day.

Several studies have examined alterations in cholesterol and lipid content following acrolein exposure in a cardiovascular disease mouse model prone to atherosclerosis (apoE$^{-/-}$). Mice given a single gavage dose of 5 mg/kg had increased plasma cholesterol and triglycerides compared to controls, similar to what was seen in wild type mice (Conklin et al. 2010). Gavage exposure to 2.5 mg/kg/day acrolein for 8 weeks resulted in an increase in serum cholesterol and low-density lipids (Srivastava et al. 2011). Exposure to 3 mg/kg/day acrolein in drinking water for 1 month resulted in an increase in serum cholesterol, triglycerides, and lipid peroxides (Rom et al. 2017).

2.10 RENAL

No studies were located regarding renal effects in humans following exposure to acrolein by any route.

No consistent renal effects have been identified in animal studies following acrolein exposure. Exposure to 44–305 ppm acrolein for 30 minutes resulted in renal hyperemia in rats, although the severity was not described (Skog 1950). Slightly increased relative kidney weights (6–18% were observed following intermediate-duration inhalation exposure to 4.0 or 4.9 ppm in rats and hamsters (Feron et al. 1978; NTP 1981), but body weights were decreased at the same exposure concentrations. Most intermediate- and chronic-duration studies in rats, mice, rabbits, and other species have not observed similar weight differences and no associated histopathology was found in any species (Conklin et al. 2017b; Feron and Kruysse 1977; Feron et al. 1978; Kutzman et al. 1984; Matsumoto et al. 2021).

Following intermediate-duration oral exposure, increased urea nitrogen was observed in rats gavaged for 14 weeks, although the study authors suggested a non-renal cause (Auerbach et al. 2008; NTP 2006a). Histopathological examination of the renal system (i.e., kidneys, bladder) revealed no effects after acute-duration oral exposure to acrolein in rats (Sakata et al. 1989), intermediate-duration exposure in rats or mice (Auerbach et al. 2008; NTP 2006a; Parent et al. 1992c), or chronic-duration exposure in rats (Parent et al. 1992a), mice (Parent et al. 1991a), or dogs (Parent et al. 1992b). Sakata et al. (1989) was not included in Table 2-2 or plotted in Figure 2-3 because a control group was not included. Negative results were also obtained from the urinalysis of mice after single gavage of up to 5 mg/kg (Conklin et al. 2010) and in dogs exposed to up 2 mg/kg/day for 53 weeks (Parent et al. 1992b).

2.11 DERMAL

Very few studies have assessed the potential dermal effects of acrolein exposure. Two aquatic pesticide workers experienced skin irritation and burns following occupational exposure to acrolein (CDC 2013). Volunteers receiving topical applications of ≥1% solution of acrolein in ethanol exhibited evidence of dermal irritation, and a 10% solution resulted in papillary edema, polymorphonuclear infiltrates, and epidermal necrosis 48 hours after exposure (Lacroix et al. 1976). In an occupational accident, an employee sprayed in the face with a high concentration of acrolein experienced burns to his checks and eyelids and edema of the eyelids which reduced the palpebral opening to a few millimeters (Champeix et al. 1966). This study was not included in Table 2-3 because the exposure concentration was not known.

Histopathological examination of the external skin revealed no effects after chronic-duration inhalation exposure to acrolein in mice and rats (Matsumoto et al. 2021), intermediate-duration oral exposure in rats or mice (Auerbach et al. 2008; NTP 2006a), or chronic-duration orally exposed mice (Parent et al. 1991a), or rats (Parent et al. 1992a). However, scattered areas of dermatitis were reported in two of six female dogs exposed to 2 mg/kg/day for 53 weeks (Parent et al. 1992b).

2.12 OCULAR

Eye irritation appears to be a sensitive effect of airborne acrolein and a more sensitive effect than nose or throat irritation. An aquatic pesticide worker experienced burning, watery eyes immediately following exposure to an unknown amount of acrolein, while five additional workers also exhibited eye irritation (CDC 2013). Volunteers reported eye irritation in a 90-second exposure to 0.6 ppm acrolein (following prior exposure to 0.15, 0.30, and 0.45 ppm; 8-minute recovery between exposures), while exposure to gradually increasing acrolein levels revealed that acrolein concentrations ≥0.26 ppm for 40 minutes resulted in irritation, measured by increasing eye blink frequency and subjective reporting (Weber-Tschopp et al. 1977). Blink rate peaked at 0.5 ppm and decreased at the higher dose. These experiments were not presented in the LSE table due to changing exposure concentrations over time (continuous exposure) or exposure of the same subjects to discrete short-term increasing exposure concentrations (discrete exposure). The irritation response was reported to be stronger for continuous exposure compared to discrete exposure at the same concentration, suggesting that irritation severity increases over time with cumulative exposure (Weber-Tschopp et al. 1977). Eye irritation from a 60-minute, 0.3-ppm exposure was greater than nose and throat irritation and was scored by participants as “a little” at 10 minutes and “medium” at 40 minutes with no further increase in severity. At 40 minutes, the

respective nose and throat irritation scores were "a little" and "not at all" (Weber-Tschopp et al. 1977). In other studies, lacrimation occurred within 20 seconds in individuals exposed to 0.81 ppm, and within 5 seconds at 1.22 ppm (Sim and Pattle 1957), while eye irritation, measured by eye blink frequency and subjective reporting, was reported following 100 minutes of exposure to 0.11 ppm (Dwivedi et al. 2015).

The ocular effects observed in experimental animals are qualitatively similar to those described in humans. Direct liquid or vapor application of 30 µL into the eyes of rabbits caused severe eyelid swelling and inflammation, corneal opacity, excessive tear secretion, and corneal edema (Gupta et al. 2020). Exposure to vapors generated after 10 µL of acrolein was applied to a filter paper disc and then placed in a glass goggle resulted in corneal erosions in rabbit eyes (Dachir et al. 2015). Vapor concentrations of 0.7–3.7 ppm acrolein caused eye irritation in dogs and monkeys as evidenced by lacrimation, excessive salivation, and closing of the eyes, but guinea pigs and rats appeared to be less sensitive, since concentrations up to 3.7 ppm had no noticeable effect in these species (Lyon et al. 1970). At 4.9 ppm, rats, hamsters, and rabbits kept their eyes closed most of the time, hamsters salivated with nasal drainage, and rabbits had respiratory difficulty and sneezed (Feron et al. 1978). Severe eye irritation was reported in rats exposed to 12 ppm for 4 hours (Murphy et al. 1964) and lacrimation was observed in rats exposed to 14 ppm for 1 hour and to 4.8 ppm for 4 hours (Ballantyne et al. 1989). It is important to note that humans have the ability to articulate feelings of irritation, while in rodents, signs of irritation are blinking, closing eyes, and drainage; therefore, it is unclear if humans were, in fact, more sensitive despite lacrimation at concentrations of 0.81 ppm, compared with ≥3.7ppm in animals.

Increased discharge from the eyes was observed in rats orally administered 10 mg/kg/day for 14 weeks (Auerbach et al. 2008; NTP 2006a). Histopathological examination of the eyes revealed no effects after intermediate-duration oral exposure to acrolein in rats or mice (Auerbach et al. 2008; NTP 2006a) or chronic-duration exposure in rats (Parent et al. 1992a), mice (Parent et al. 1991a), or dogs (Parent et al. 1992b).

2.13 ENDOCRINE

No studies were located regarding endocrine effects in humans following exposure to acrolein by any route.

Data on potential endocrine effects in animals are also limited. Plasma corticosterone was increased in rats exposed to 4 ppm acrolein over 1 or 2 days (Snow et al. 2017). Increased adrenal weights have been

reported in rats exposed to 6.4 ppm for 4 hours (Murphy et al. 1964) or to 4.9 ppm for 13 weeks (Feron et al. 1978), although no differences were observed in similarly exposed hamsters or rabbits, and no associated histopathology was found in any species. Exposure for 2 years (6 hours/day, 5 days/week) did not affect weights or presence of non-neoplastic lesions in the adrenals, pituitary, or thyroid glands in mice or rats (Matsumoto et al. 2021).

Histopathological examination of the endocrine system (i.e., thyroid, parathyroid, pituitary, adrenals) revealed no effects after intermediate-duration oral exposure to acrolein in rats or mice (Auerbach et al. 2008; NTP 2006a; Parent et al. 1992c) or chronic-duration exposure in rats (Parent et al. 1992a), mice (Parent et al. 1991a), or dogs (Parent et al. 1992b).

2.14 IMMUNOLOGICAL

Data on the potential immunological effects of acrolein in humans are extremely limited. A human controlled exposure study found no differences in inflammatory markers in the serum (IL-6) and sputum (IL-6, IL-8) of volunteers exposed to 0.11 ppm for 2 hours (Dwivedi et al. 2015).

No histological changes have been reported in immune organs following inhalation exposure to acrolein in animals. No histopathological changes were noted in the spleens of rats exposed to 44–305 ppm acrolein for 30 minutes (Skog 1950) and exposure to 5 ppm acrolein for 3 days did not result in an inflammatory response in the lung of mice (Kasahara et al. 2008). In mice exposed to acrolein for 6 hours/day, 5 days/week, for 12 weeks, there were multiple changes in circulating blood immune cells (Conklin et al 2017b). These changes include decreased granulocytes and CD8+ T cells at 1 ppm, a dose-related decrease in CD 11b+ monocytes, and decreased CD19+ B-cells at both doses, but not dose related. Changes in natural killer and CD4+ T-cells were observed at the low dose, but not the high dose. A significant 20% reduction in spleen weight was reported for exposure of rats to 3 ppm intermittently for 3 weeks, but the effect was not apparent when normalized to final body weight (Leach et al 1987). No changes in spleen weights or histopathology were observed in rodent intermediate- and chronic-duration inhalation studies with concentrations up to 5 ppm (Conklin et al. 2017b; Feron and Kruysse 1977; Feron et al. 1978; Kutzman et al. 1984; Matsumoto et al. 2021; NTP 1981). Relative thymus weights were decreased in rats exposed to 4.9 ppm for 13 weeks, but the study authors considered the effect to be associated with reduced body weight gain rather than a result of treatment (Feron et al. 1978). No differences in thymus weight were observed in similarly exposed hamsters, and no associated histopathology was found in any species (Feron et al. 1978).

Decreased thymus weights, thymocyte atrophy and necrosis, and lymphoid follicular cell depletion in the spleen were observed in rats gavaged with 10 mg/kg/day for 14 weeks (Auerbach et al. 2008; NTP 2006a). Atrophy and necrosis in the thymus, necrosis in the mandibular and mesenteric lymph nodes, and depletion of the lymphoid follicle in the spleen were seen in mice gavaged with 20 mg/kg/day. In contrast, histopathological examination of the immunological system (i.e., spleen, thymus, lymph nodes) revealed no effects after acute-duration oral exposure to acrolein in rats (Sakata et al. 1989), intermediate-duration exposure in rats (Parent et al. 1992c), or chronic-duration exposure in rats (Parent et al. 1992a), mice (Parent et al. 1991a), or dogs (Parent et al. 1992b). Sakata et al. (1989) was not included in Table 2-2 or plotted in Figure 2-3 because a control group was not included.

Several studies have evaluated the effects of acrolein exposure on immune function or inflammatory response, particularly in the respiratory system, although the results have not been conclusive. A single 3-hour inhalation exposure of 0.09 ppm in mice had no impact on bactericidal activity in response to *Klebsiella pneumoniae*, although repeated exposures over 5 days resulted in lower removal by alveolar macrophages (Aranyi et al. 1986). In rats exposed to acrolein concentrations up to 3 ppm for 6 hours/day, 5 days/week for 3 weeks, no effect was observed on macrophage function in response to *K. pneumoniae* (Sherwood et al. 1986). Clearance of intrapulmonary *Staphylococcus aureus* was reduced in mice following acrolein exposure to ≥3 ppm for 8 hours; this impairment was exacerbated in mice pre-infected with Influenza A virus (Astry and Jakab 1983). Acrolein exposure (5 ppm for 6 hours/day for 3 days) in conjunction with instillation of lipopolysaccharide (LPS) (*Escherichia coli*) did not alter the inflammatory response in mice induced by LPS alone (Kasahara et al. 2008). Rats exposed to 0.55 ppm for 10–26 days (but not 60–180 days) had significantly lower numbers of alveolar macrophages, and additional respiratory challenge with *Salmonella enteritidis* revealed that acrolein-exposed animals were more susceptible to bacterial-induced mortality (Bouley et al. 1975). In contrast, rats exposed to 3 ppm acrolein for 3 weeks were not more susceptible to mortality following intravenous (i.v.) exposure to *Listeria monocytogenes* (Leach et al. 1987).

Mixed results have been observed in the nasal or bronchoalveolar lavage fluid (BALF) parameters in rodents sensitized and/or challenged with ovalbumin (OVA) in conjunction with acrolein exposure. Inflammatory cells (neutrophils and macrophages) were increased in the bronchoalveolar fluid of mice exposed to 5 ppm acrolein for 10 minutes, and this increase was even greater (and included eosinophils) in OVA-sensitized mice (Kim et al. 2019). Mice exposed to 5 ppm acrolein for 4 days showed a suppressed inflammatory response in the nose and lungs, measured as a decrease in inflammatory cell

infiltrates and IL-12p40 levels, compared to challenge only (Spiess et al. 2013), while another study found an increase in neutrophils in mice exposed to 5 ppm for 2 weeks (O'Brien et al. 2016). Suppression of the inflammatory response, measured as a reduction in airway IL-33, IL-25, and IL-1α levels, was also observed in mice challenged with the airborne allergen house dust mite (Danyal et al. 2016).

Mechanisms. The mechanisms of acrolein effects are discussed in detail in Section 2.21, Mechanisms of Toxicity. Acrolein's effect on the immune system is expected to be mediated by its effects on immune signaling proteins. In a review of acrolein mechanisms, Moghe et al. (2015) proposed that acrolein could suppress immune responses by inhibiting macrophage function through inhibition of NF-κB, by alkylation of immune signaling proteins, or by tipping the balance of inflammatory mediators in favor of anti-inflammatory responses. For example, *in vitro* studies using human T cells showed that acrolein could directly alkylate amino acids in NF-κB, leading to reduced binding to proinflammatory mediators (IL-2, IL-10, TNFα, granulocyte-macrophage colony stimulating factor [GMCSF], and IFN-γ) (Moghe et al. 2015). The effects of acrolein on the immune system may depend upon dose and/or exposure duration. Moghe et al. (2015) postulated that acute, high-level exposures were more likely to suppress the immune response, while prolonged, low-level exposures would increase inflammation. Additional information on the evidence for acrolein-induced inflammation is presented in Section 2.21.

2.15 NEUROLOGICAL

Few studies have evaluated the potential neurological effects of acrolein exposure in humans. An aquatic pesticide worker was diagnosed with lateral medullary syndrome and experienced dysphagia and facial droop following exposure to an unknown amount of acrolein, while three additional workers also reported headaches (CDC 2013). In a case-control study, no associations were observed between the urinary acrolein metabolite, 3-HPMA, and attention-deficit hyperactivity disorder in a group of children in Taiwan (Waits et al. 2022).

Nonspecific inflammatory changes were reported in the brains of rats, dogs, monkeys, and guinea pigs after a continuous 90-day exposure to 1.8 ppm acrolein (Lyon et al. 1970); however, the nature and severity of these lesions was not described. No histopathological changes were noted in the brains of rats exposed to 44–305 ppm acrolein for 30 minutes (Skog 1950). Increased relative brain weights have frequently been observed following intermediate- and chronic-duration inhalation exposure in rats and hamsters at concentrations ≥1.4 ppm (Feron and Kruysse 1977; Feron et al. 1978; Kutzman et al. 1984;

NTP 1981), but only at exposure levels at which body weights were decreased. No differences in brain weights were observed in similarly exposed rabbits (Feron et al. 1978), and no associated histopathology was found in any species. Neurobehavioral tests have shown mixed results following inhalation exposure to acrolein. Rats exposed to 0.3 ppm acrolein for 4 days showed altered pain thresholds and spent longer times in corners (Kunkler et al. 2018), while no differences in behavioral measurements (exploratory behavior and locomotive activity) were observed in rats exposed up to 4 ppm for 62 days (Kutzman et al. 1984). Feron et al. (1978) reported clinical signs of toxicity in animals exposed to ≥1.4 ppm for 13 weeks, including hyperactivity followed by somnolence in rats and hamsters and sneezing in rabbits. At 4.9 ppm, additional signs included eyes closed in all animals, salivation and nasal discharge in hamsters, occasional breathing difficulty in rabbits, and piloerection in rats. Many or all of these clinical signs may be attributable to respiratory irritation/inflammation and subsequent hypoxia induced by acrolein.

Neurobehavioral tests in rats and mice following acute- and intermediate-duration oral exposures to acrolein concentrations ≥2.5 mg/kg/day have found decreased response to tail pinch and loss of elevation reflexes/poor body tone (Sprince et al. 1979), decreased rotarod latency (Chen et al. 2019), and increased escape latency in the Morris water maze test (Huang et al. 2013). Histopathological examination in one study revealed neuronal loss and inflammation in the hippocampus in rats gavaged for 8 weeks at 2.5 mg/kg/day (Huang et al. 2013), (the other two neurobehavioral studies examined the brain for histopathology). Histopathological examination of the neurological system (i.e., brain, spinal cord, nerves) revealed no effects after intermediate-duration oral exposure to acrolein in rats or mice (Auerbach et al. 2008; NTP 2006a; Parent et al. 1992c) or chronic-duration exposure in rats (Parent et al. 1992a), mice (Parent et al. 1991a), or dogs (Parent et al. 1992b).

2.16 REPRODUCTIVE

No studies were located regarding reproductive effects in humans after exposure to acrolein by any route.

No evidence of reproductive toxicity has been found in animals exposed to acrolein by inhalation. Reproductive fitness, measured as the number of pregnant rats, corpora lutea, number of viable fetuses, and preimplantation loss, was not affected by intermediate-duration inhalation exposure to acrolein (Bouley et al. 1975; NTP 1981). Increased relative testis and ovary weights (≤15–20%) have been reported in rats and hamsters exposed to acrolein concentrations of 0.55 and 4.9 ppm, but these changes occurred in the context of reduced body weights (Bouley et al. 1975; Feron et al. 1978). No differences

were observed in similarly exposed mice or rabbits, and no associated histopathology or alterations to sperm quality have been found in any species (Feron and Kruysse 1977; Feron et al. 1978; Matsumoto et al. 2021; NTP 1981).

Similarly, no evidence of reproductive toxicity has been found in animals following oral exposure to acrolein. Reproductive performance was not affected in 2 generations of rats gavaged up to 7.2 mg/kg/day acrolein (Parent et al. 1992c). No differences in premature deliveries or spontaneous abortions were observed in rabbits gavaged with up to 2 mg/kg/day on GDs 7–19 (Parent et al. 1993). Histopathological examination of the reproductive system (i.e., testes, ovaries, epididymides, uterus, cervix) revealed no effects after intermediate-duration oral exposure to acrolein in rats or mice (Auerbach et al. 2008; NTP 2006a; Parent et al. 1992c) or chronic-duration exposure in rats (Parent et al. 1992a), mice (Parent et al. 1991a), or dogs (Parent et al. 1992b).

2.17 DEVELOPMENTAL

No studies were located regarding developmental effects in humans following exposure to acrolein from any route.

Only a single study was identified that evaluated developmental effects in animals after inhalation exposure to acrolein. No effects on fetal number or body weight were observed in male and female rats exposed continuously for 26 days (3 days prior to mating and presumed GDs 0–22) to 0.55 ppm (Bouley et al. 1975).

Alterations in fetal weight and skeletal abnormalities have been observed following oral exposure to acrolein in animals. Decreased pup weight (7% at postnatal day [PND] 21 in F1 generation) was observed in a 2-generation study in rats gavaged with 6 mg/kg/day (Parent et al. 1992c). Increased skeletal anomalies, including incomplete ossification of the skull, vertebrae, metacarpals, and metatarsals, were seen in rat fetuses when dams were gavaged with 10 mg/kg/day acrolein on GDs 7–19 (EPA 1983). However, in both of these studies, maternal toxicity was also observed at the dose level that adverse effects were seen in pups. No other evidence of developmental toxicity, such as number of implantations, gestation length, resorptions, or live fetuses per litter, were observed in rats or rabbits gavaged with up to 10 mg/kg/day during gestation (EPA 1983; Parent et al. 1992c, 1993).

2.18 OTHER NONCANCER

Human data on other noncancer effects related to acrolein exposure are limited. In a population-based study, associations were observed between urinary acrolein metabolites and the prevalence of diabetes and insulin resistance (Feroe et al. 2016).

Animal studies have identified potential metabolic effects following inhalation or oral exposure to acrolein. Body temperature was decreased in mice exposed to 250 ppm acrolein for 30 minutes (Conklin et al. 2017a). Glucose tolerance was altered in rats exposed nose-only to 4 ppm acrolein over 1 or 2 days (Snow et al. 2017). In contrast, no differences in blood glucose, insulin, or glucose tolerance were observed in mice exposed by inhalation to 1 ppm acrolein for 12 weeks (Conklin et al. 2017b). Following oral exposure, blood glucose and insulin were increased in mice gavaged with 2.5 mg/kg/day for 4 weeks, resulting in impaired glucose tolerance (Wang et al. 2021).

2.19 CANCER

IARC (2021) reviewed six epidemiology studies that evaluated the relationship between exposure to acrolein and cancer (one cohort study, two case-control studies, and three nested case-control studies). These studies were considered uninformative, because they were either mechanistic in nature or had poor study design and exposure assessment.

Mixed results have been observed in animal studies evaluating the carcinogenic effects of acrolein inhalation. Feron and Kruysse (1977) exposed hamsters to 4.0 ppm acrolein for 52 weeks and found no evidence of respiratory tract tumors or tumors in other tissues and organs, although epithelial hyperplasia and metaplasia were observed in the nasal cavity. However, this study is considered to be of too short duration to determine carcinogenicity, and the maximum tolerable dose (MTD) of acrolein may not have been achieved in this study. In a more recent study by Matsumoto et al. (2021), increased incidence of nasal tumors was observed in female rats (rhabdomyomas, 8%) and female mice (adenomas, 32%) exposed to 2 and 1.6 ppm acrolein, respectively, for up to 2 years, although similar results were not observed in male rats or male mice. There is no clear mode of action that would lead to the observed sex-based differences.

Questionable evidence of the carcinogenicity of acrolein in animals is provided by a few long-term oral studies. Lijinsky and Reuber (1987) reported a cancer bioassay in which groups of rats were given

acrolein in the drinking water at concentrations up to 36 mg/kg/day for 104–124 weeks. This study had several limitations including issues with stability of the acrolein solutions (reported loss of 18% after 6 days at 5°C, 27% loss after 3 days at 22°C), water consumption was not measured/reported, and treatments had to be stopped due to the animals' refusal to drink the solution. The only indication of a carcinogenic effect of acrolein was the incidence of neoplasms of the adrenal cortex in high-dose female rats (5/20 adenomas, 2/20 hyperplastic nodules). Additional oral studies have failed to detect significant cancer incidence in animals. Gavage treatment of rats with up to 2.5 mg/kg/day for 102 weeks failed to produce tumor incidences, including adrenal tumors, which were significantly different from controls (Parent et al. 1992a). Extensive histopathological examination did not reveal any carcinogenic effects in mice (Parent et al. 1991a) or dogs (Parent et al. 1992b) after oral exposure to 4.5 or 2 mg/kg/day acrolein, respectively, for 12–18 months. Because of the disparate results of the Lijinsky and Reuber (1987) and Parent et al. (1991a, 1992a) studies, an independent pathology working group (PWG) re-evaluated the Lijinsky and Reuber tumor data (cited in Parent et al. 1992a). The PWG concluded that the incidence of cortical tumors in treated females was within the limits of historical controls and were of no biological significance for adrenal cancer from acrolein exposure.

The HHS has not classified acrolein as to its carcinogenicity. IARC has classified acrolein as "probably carcinogenic to humans" (Group 2A) based on "sufficient" evidence of carcinogenicity in experimental animals and "strong" mechanistic evidence (IARC 2021). EPA concluded that the potential carcinogenicity of acrolein cannot be determined because the existing "data are inadequate for an assessment of human carcinogenic potential for either the oral or inhalation route of exposure" (IRIS 2003).

Mechanisms. IARC (2021) provided a comprehensive review of the cancer mechanistic data on acrolein structured around the 10 key characteristics of carcinogenicity (Smith et al. 2016). In their review, IARC (2021) emphasized acrolein's electrophilicity and capacity to bind both deoxyribonucleic acid (DNA) and proteins; its genotoxicity and ability to alter DNA repair; its ability to induce oxidative stress and inflammation; its suppression of immune responses; and its ability to alter cell proliferation. The study authors did not provide any specific links between the mechanistic data and the tumors seen in animal studies of acrolein carcinogenicity; however, the observation of nasal tumors in rats and mice exposed to acrolein by inhalation (Matsumoto et al. 2021) is consistent with the greater uptake of acrolein in the upper respiratory tract (see Section 3.1) and the evidence for oxidative stress, inflammation, and altered cell proliferation (hyperplasia, metaplasia, and dysplasia) in the nasal cavity of rats exposed by inhalation

(IARC 2021). Further information on the molecular mechanisms of acrolein toxicity and carcinogenicity is provided in Section 2.20 (Genotoxicity) and Section 2.21 (Mechanisms of Toxicity).

2.20 GENOTOXICITY

No studies were located regarding the genotoxic effects of acrolein in humans or animals following inhalation, oral, or dermal exposure. Acrolein was found to be non-mutagenic *in vivo,* as judged by the dominant lethal assay in the mouse (Epstein et al. 1972), the micronucleus assay in mice peripheral blood (NTP 2006a), and the sex-linked recessive lethal test in *Drosophila* (Zimmering et al. 1985).

The *in vitro* genotoxicity of acrolein has been investigated in prokaryotic and eukaryotic organisms and in mammalian cell systems (Table 2-8). In prokaryotic cells, the overall evidence, indicates that acrolein is weakly mutagenic without activating systems and non-mutagenic in the presence of activating systems in *Salmonella typhimurium* and *Escherichia coli* (see Table 2-8 for references). In the yeast, *Saccharomyces cerevisiae*, acrolein was not mutagenic without activating systems (Izard 1973).

Table 2-8. Genotoxicity of Acrolein *In Vitro*

		Results		
		Activation		
Species (test system)	Endpoint	With	Without	Reference
Prokaryotic organisms				
Salmonella typhimurium	Reverse mutation	–	–	Andersen et al. 1972
S. typhimurium	Reverse mutation	–	–	Florin et al. 1980
S. typhimurium	Reverse mutation	–	–	Loquet et al. 1981
S. typhimurium	Reverse mutation	–	–	Bignami et al. 1977
S. typhimurium	Reverse mutation	–	(+)	Lijinsky and Andrews 1980
S. typhimurium	Reverse mutation	–	+	Lutz et al. 1982
S. typhimurium	Reverse mutation	–	+	Eder et al. 1982
S. typhimurium	Reverse mutation	–	–	Basu and Marnett 1984
S. typhimurium	Reverse mutation	ND		Bartsch et al. 1980
S. typhimurium	Reverse mutation	ND	(+)	Khudoley et al. 1987
S. typhimurium TA1535, TA1537, TA1538	Reverse mutation	–	–	Parent et al. 1996b
S. typhimurium TA98	Reverse mutation	–	(+)	Parent et al. 1996b
S. typhimurium TA100	Reverse mutation	+	+	Parent et al. 1996b

Table 2-8. Genotoxicity of Acrolein *In Vitro*

Species (test system)	Endpoint	Results		Reference
		Activation		
		With	Without	
S. typhimurium	Reverse mutation	ND	+	Marnett et al. 1985
S. typhimurium	Reverse mutation	ND	+	Foiles et al. 1989
S. typhimurium (vapor assay in sealed desiccator)	Reverse mutation	–	–	NTP 2006a
S. typhimurium (preincubation) TA98, TA1535, TA1538	Reverse mutation	–	–	NTP 2006a
S. typhimurium (preincubation) TA100	Reverse mutation	(+)	–	NTP 2006a
S. typhimurium TA1535	Reverse mutation	–	(+)	Waegemaekers and Bensink 1984
Escherichia coli PQ37	Reverse mutation	–	–	von der Hude et al. 1988
E. coli K-12/343/133	Reverse mutation	–	ND	Ellenberger and Mohn 1977
E. coli WP2uvrA	Reverse mutation	–	(+)	Parent et al. 1996b
E. coli WPuvrA	Reverse mutation	ND	(+)	Hemminki et al. 1980
E. coli WPuvrA	Reverse mutation	ND	+	Bilimoria 1975
E. coli AB1157	Reverse mutation	–	ND	VanderVeen et al. 2001
Non-mammalian eukaryotic cells				
Saccharomyces cerevisiae	Gene mutation	ND	–	Izard 1973
S. cerevisiae MB1072-2B	Chromosomal aberrations	ND	–	Fleer and Brendel 1982
Mammalian cells				
Human fibroblasts (normal)	Gene mutation	No data	–	Curren et al. 1988
Human fibroblasts (cells deficient in DNA repair)	Gene mutation	No data	+	Curren et al. 1988
Human fibroblast	Gene mutation	ND	+	Kawanishi et al. 1998
Human fibroblast	Gene mutation	ND	–	Kim et al. 2007
Mouse embryonic fibroblast	Gene mutation	ND	–	Kim et al. 2007
Chinese hamster ovary cells	Gene mutation	–	–	Parent et al. 1991b
Chinese hamster V79 cells	Gene mutation	ND	+	Smith et al. 1990
Human myeloid cells K562	DNA damage	ND	+	Crook et al. 1986
Human bronchial cells	DNA damage	ND	+	Grafstrom et al. 1988
Human pulmonary epithelial cell (A549)	DNA damage	ND	+	Wang et al. 2017
Human lung fibroblast (MRC-5)	DNA damage	ND	+	Wang et al. 2017
Human bronchial cells	Impaired DNA repair	ND	+	Krokan et al. 1985

Table 2-8. Genotoxicity of Acrolein *In Vitro*

Species (test system)	Endpoint	Results		Reference
		Activation		
		With	Without	
Human bladder cells (UROtsa)	Impaired DNA repair	ND	+	Lee et al. 2014
Human bronchial epithelial	Impaired DNA repair	ND	+	Wang et al. 2012
Human lung fibroblast	Impaired DNA repair	ND	+	Wang et al. 2012
Human hepatoma line (HepG2)	DNA strand breaks	ND	+	Li et al. 2008
Leydig cells	DNA strand breaks	ND	+	Yildizbayrak et al. 2020
Human Pulmonary epithelial cells	DNA strand breaks	ND	+	Zhang et al. 2018
Bronchial epithelial cells (BEAS-2B)	DNA strand breaks	ND	+	Zhang et al. 2020a
Chinese hamster ovary cells	DNA strand breaks	+	+	Au et al. 1980
Chinese hamster ovary cells	Sister chromatid exchange	+	+	Au et al. 1980
Chinese hamster ovary cells	Sister chromatid exchange	–	+	NTP 2006a
Chinese hamster ovary cells	Chromosomal aberrations	–	–	NTP 2006a
Human epithelial cell line (HT-29)	DNA adduct	ND	+	Pan et al. 2012
Human bronchial epithelial	DNA adduct	ND	+	Wang et al. 2012
Human lung fibroblast	DNA adduct	ND	+	Wang et al. 2012
Mouse embryonic fibroblast	DNA adduct	ND	+	Kim et al. 2007
Acellular systems				
Calf-thymus DNA	DNA adduct	ND	+	Kozekov et al. 2010
Calf-thymus DNA	DNA adduct	ND	+	Pawłowicz and Kronberg 2008
Calf-thymus DNA	DNA adduct	ND	+	Pawlowicz et al. 2006

+ = positive results; (+) = weakly positive results; – = negative results; DNA = deoxyribonucleic acid; ND = not determined

In mammalian cells, acrolein exposure resulted in DNA damage and adduct formation and impaired DNA repair in the absence of activating systems (Table 2-8). Acrolein was found to be non-mutagenic to normal human fibroblasts, mouse embryonic fibroblasts, and Chinese hamster ovary cells (Curren et al. 1988; Kim et al. 2007; Parent et al. 1991b) in culture; however, positive mutagenic responses were observed in fibroblasts with a deficient DNA repair system (Curren et al. 1988). DNA base substitutions and intra-strand cross-links were observed in human fibroblasts containing shuttle vector plasmids bearing the *supF* marker gene (Kawanishi et al. 1998). Acrolein exposure reduced DNA repair capabilities in human bronchial cells, bladder cells, and lung fibroblasts (Krokan et al. 1985; Lee et al.

2014; Wang et al. 2012). Acrolein inhibited the activity of DNA polymerase as well as DNA and ribonucleic acid (RNA) synthesis in rat liver cell nuclei (Crook et al. 1986; Grafstrom et al. 1988; Krokan et al. 1985) and is a potent inhibitor of the DNA repair enzyme, 0_6-methylguanine-DNA methyl transferase. Acrolein induced chromosome breakage and sister-chromatid exchange in Chinese hamster ovary cells (Au et al. 1980). DNA damage was seen in human myeloid cells, pulmonary epithelial cells, bronchial epithelial cells, Leydig cells, and lung fibroblasts in culture following acrolein exposure (Crook et al. 1986; Grafstrom et al. 1988; Li et al. 2008; Wang et al. 2017; Yildizbayrak et al. 2020; Zhang et al. 2018, 2020a). The mechanism by which acrolein induces genotoxicity in mammalian cells is not known, but it has been shown that acrolein can form adducts with DNA, such as alpha-hydroxypropano-2'deoxyguanosine and gamma-hydroxypropano-2'-deoxyguanosine in mouse embryonic fibroblast cells (Kim et al. 2007), human epithelial cells (Pan et al. 2012), human bronchial epithelia and lung fibroblasts (Tang et al. 2011), and calf-thymus DNA (Kozekov et al. 2010; Pawłowicz and Kronberg 2008; Pawlowicz et al. 2006). Yang et al. (2002) showed that acrolein adduction to DNA may be insignificant for the introduction of miscoding errors, as translesion DNA synthesis was high and miscoding incidence was <1% in human HeLa and xeroderma pigmentosum cells. The same inability of acrolein DNA adducts to cause miscoding was observed in *E. coli* as well (VanderVeen et al. 2001). Because of the limited number of *in vivo* tests, there is insufficient evidence to predict that acrolein poses a genotoxic threat to humans.

Overall, *in vitro* data showed weak mutagenic potential of acrolein in bacterial and mammalian cells without metabolic activation (Table 2-8). Acrolein produces DNA adducts and DNA damage and inhibits DNA repair in mammalian cells (Table 2-8). The mechanism of these changes is not clear but may involve downregulation of Werner's syndrome protein (WRN). This protein has been shown to be involved in DNA repair, telomere maintenance, and cellular senescence (Szekely et al. 2005). Jang et al. (2014) exposed normal human lung fibroblasts (NHLFs) *in vitro* to acrolein and observed a downregulation of WRN protein and an increase in acrolein-induced telomere attrition and cellular senescence.

2.21 MECHANISMS OF TOXICITY

The mechanisms of acrolein toxicity have been extensively reviewed (IARC 2021; Moghe et al. 2015); the discussion here is based on these reviews. Many of the toxic effects of acrolein result from the same molecular initiating event: irreversible binding to cellular proteins and macromolecules. As a highly reactive electrophile, acrolein readily interacts (through Michael addition and/or Schiff base cross-

linking) with biological nucleophiles including the sulfhydryl group of cysteine, amino group of lysine, and imidazole group of histidine (Moghe et al. 2015). These amino acid targets are incorporated into a wide variety of proteins that are important for enzyme catalysis, redox signaling, cytoskeletal components, reactive oxygen species sensing, cellular buffering, and other cellular processes. Adduction of these proteins alters their functioning, leading to cellular-level perturbations including mitochondrial dysfunction, disrupted signal transduction, oxidative stress, inflammation, endoplasmic reticulum (ER) stress, and damage to membrane integrity or cellular structure. Protein modification by acrolein may also alter physiological responses to other toxicants by irreversibly altering xenobiotic-metabolizing enzymes such as arylamine N-acetyltransferases. Because of the wide range of cellular functions affected by acrolein protein adduction, this chemical can damage virtually any organ; however, because it is so reactive, its systemic distribution is often limited and its toxic effects tend to be most severe in the tissues acrolein first contacts (e.g., respiratory tract after inhalation exposure, gastrointestinal tract after oral exposure, and skin after dermal exposure).

Oxidative Stress. In laboratory rodents exposed to acrolein by inhalation, gavage, and/or intraperitoneal injection, glutathione depletion has been seen in the liver, nasal cavity, tracheobronchial mucosa, and lungs (IARC 2021). At low exposure levels, cellular thiol-containing antioxidants such as glutathione may bind to and detoxify acrolein; however, as acrolein dose increases and glutathione is depleted, oxidative stress and tissue damage ensues. Additional evidence for acrolein-induced oxidative stress in exposed rodents includes decreased total antioxidant capacity and increased lipid peroxidation (measured as levels of 8-isoprostane or thiobarbituric acid [TBARS]) in the spleen and thymus of rats and livers of mice exposed to acrolein (IARC 2021). Acrolein both induces and results from lipid peroxidation; in fact, lipid peroxidation is considered to be the major source of endogenous acrolein production (Burcham 2017). *In vitro* studies provide support for the association between acrolein exposure and oxidative stress. Depletion of glutathione and antioxidant enzymes (superoxide dismutase and glutathione peroxidase) was observed in human retinal epithelial cells incubated with acrolein; increased generation of oxygen radical was observed in exposed bovine pulmonary arterial endothelial cells; and supplementation of cell medium with antioxidants mitigated the toxicity of acrolein on liver cells (IARC 2021; Moghe et al. 2015).

Endoplasmic Reticulum Stress. ER stress is believed to play a role in several diseases, including neurodegenerative, cardiovascular, respiratory, and liver diseases, as well as cancer (Moghe et al. 2015). The cellular response to ER stress is the unfolded protein response (UPR), a complex signal transduction pathway aimed at reducing the load of unfolded proteins and restoring or maintaining cell function. Under chronic ER stress conditions, apoptosis is triggered. Acrolein adduction of proteins is expected to

cause ER stress, and both ER stress and the UPR have been observed *in vitro* in endothelial cells, hepatocytes, and Swiss 3T3 cells incubated with acrolein. Further, intraperitoneal injection of rats with acrolein was shown to induce ER stress and apoptosis in the lungs, as well as emphysematous changes in the lung (Moghe et al. 2015).

Mitochondrial Dysfunction. An important function of mitochondria is initiating cell signaling pathways leading to apoptosis. Depending on the dose and cell system, acrolein may induce or inhibit apoptosis. In human neuroblastoma cells and A549 cells, acrolein induced caspase-dependent and caspase-independent apoptosis (respectively), while in murine proB lymphocytes and B lymphoblastoid SKW6.4 cells acrolein exposure resulted in alkylation of caspase active sites and inhibition of apoptosis (Moghe et al. 2015). In addition to interfering with apoptotic pathways, acrolein exposure may interfere with cellular respiration. Exposure of rat liver mitochondria to acrolein resulted in dose-dependent inhibition of pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase, and complexes I and II, important components of the electron transport chain and cellular respiration (Moghe et al. 2015).

Perturbation of Signal Transduction. As with its other effects, acrolein's impact on signal transduction depends on the cell system tested and the exposure conditions. Acrolein exposure of cultured cells has resulted in activation or inactivation of protein kinases and phosphatases that regulate many cellular functions, including protein tyrosine phosphatase-1B; phosphatase PP2A; serine phosphatase; tyrosine phosphatase; and mitogen activated protein kinases (MAPKs) such as extracellular signal-regulated kinase (ERK1/2) and c-Jun N-terminal kinase (JNK) (Moghe et al. 2015). *In vivo* data to support the role of impaired signal transduction in acrolein-induced toxicity are lacking.

Impaired Membrane Structure and/or Function. Protein modification can also lead to disruptions in cell membrane integrity and function. In experiments using *ex vivo* spinal cords, exposure to acrolein resulted in increased cell membrane permeability, measured as permeation of ethidium bromide, horseradish peroxidase, and lactate dehydrogenase (LDH) levels (Moghe et al. 2015). Both *in vivo* and *in vitro* experiments showed that acrolein adducts disrupt proteins involved in presynaptic membrane neurotransmitter uptake and release. Additional evidence comes from studies showing that acrolein exposure induced structural changes in Sertoli cells (including F-actin microfilament aggregation), erythrocytes (membrane phospholipid scrambling), and bronchiolar lung cell monolayers (hyperphosphorylation of keratin-8 and ubiquitination of intermediate filaments). In animal models of multiple sclerosis and spinal cord injury, neuronal membrane and myelin damage have been correlated

with acrolein levels (Moghe et al. 2015); however, it is unclear whether acrolein induced or resulted from the damage.

Inflammation. Numerous *in vivo* bioassays have shown inflammation or increased markers of inflammation (for example, TNFα, IL-6, and IL-8), in the respiratory tract (nasal tissue, lungs, and BALF) of rats and mice exposed to acrolein by inhalation (IARC 2021). These findings are supported by an *in vivo* study in which increased levels of proinflammatory cytokines (IL-1α, IL-1β, IL-6, TNF, and IFN-γ, among others) were detected in the BALF of mice exposed to acrolein by oropharyngeal aspiration (IARC 2021; Moghe et al. 2015). *In vitro* experiments in a variety of mammalian airway cell types (human and/or rat epithelial cells, smooth muscle cells, macrophages, and fibroblasts), demonstrated that acrolein exposure activates NF-κB and upregulates proinflammatory cytokines such as IL-8 (Moghe et al. 2015). In a mast cell analog test system (RBL-2H3 cells), *in vitro* acrolein exposure resulted in degranulation (exocytosis of cytoplasmic granule contents), a process that releases a multitude of inflammatory mediators (Moghe et al. 2015).