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 nitrophenols. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

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

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

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

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 animal dermal studies are presented in Table 2-3.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects (SLOAELs) 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 important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

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

The potential health effects of nitrophenols have been evaluated in experimental animal studies only. While several epidemiological studies have evaluated potential associations between health effects and urinary levels of 4-nitrophenol, these studies measure urinary levels as a biomarker for exposure to pesticides that metabolize into 4-nitrophenol (e.g., parathion), rather than assess potential health effects of direct exposure to nitrophenols. Therefore, these studies are not discussed in this profile.

As illustrated in Figure 2-1, most of the health effects data in animals come from oral studies. Animal data are available for each exposure route and exposure duration category; however, there are limited studies available for each. Most of the studies evaluating the toxicity of nitrophenols focus on 4-nitrophenol, while only a few evaluate the toxicity of 2- or 3-nitrophenol. Many of the studies evaluating the toxicity of nitrophenols have evaluated a comprehensive set of endpoints. Lethality and body weight effects are the most examined effects in the literature, followed by reproductive and neurological effects. The genotoxicity of 2-, 3-, and 4-nitrophenol has also been examined.

Research on the health effects of nitrophenols suggest that body weight, hematological, and ocular effects are the most sensitive endpoints of toxicity:

- **Body Weight Effects.** Some studies report decreased body weight in rodents following acute-duration oral exposure to 4-nitrophenol; however, findings were inconsistent and not observed in intermediate-duration oral exposure studies. Adverse body weight effects were not observed

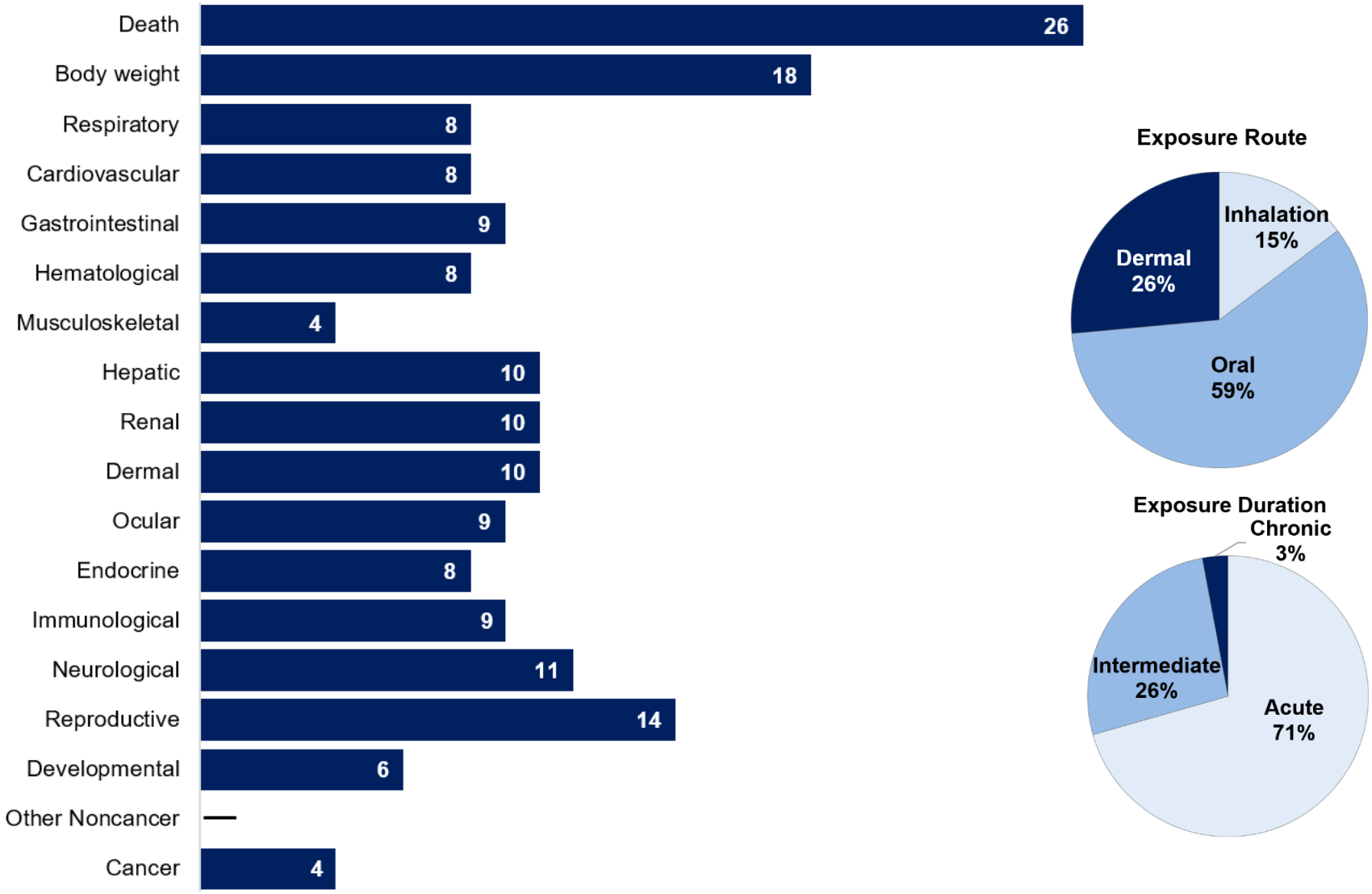
following oral exposure to 2-nitrophenol, inhalation exposure to 2- or 4-nitrophenol, or dermal exposure to 4-nitrophenol.

- **Hematological Endpoints.** Methemoglobinemia was observed in rats following acute-duration inhalation exposure to 4-nitrophenol; however, no clear evidence was observed in an intermediate-duration inhalation study in rats exposed to 4-nitrophenol. No additional hematological effects were noted after inhalation exposure to 4-nitrophenol, and no hematological effects were noted following oral exposure to 4-nitrophenol. Results of a single study investigating hematological effects in animals after 2-nitrophenol inhalation exposure did not observe any exposure-related effects.

- **Ocular Endpoints.** Ocular effects, including corneal opacity and cataracts, have been observed in rats following inhalation exposure to 4-nitrophenol. These effects are likely due to direct corneal contact with dust particles; however, a systemic effect cannot be ruled out. Direct ocular instillation of 4-nitrophenol in rabbits results in severe irritation, inflammation, corneal opacity and neovascularization, and visible destruction of the iris. No ocular effects were found following oral exposure to 4-nitrophenol. Results of a single study investigating ocular effects in animals after 2-nitrophenol inhalation exposure did not observe any exposure-related effects.

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

Most studies examined the potential lethality, body weight, neurological, and reproductive effects of nitrophenols
Studies evaluated health effects in animals only (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 33 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Smith et al. 1988									**4-nitrophenol**
1	Rat (Crl:CD) 10 M	2 weeks 5 days/week 6 hours/day (H)	0, 294, 2,133	LE, CS, BW, BC, HE, UR, GN, OW, HP	Resp	2,133			
					Cardio	2,133			
					Gastro	2,133			
					Hemato		294		Methemoglobin increased from 0.2% (in controls) to 0.87%
					Hepatic	2,133			
					Renal	2,133			
					Dermal	2,133			
					Ocular	2,133			
					Endocr	2,133			
					Immuno	2,133			
					Neuro	294	2,133		Lethargy
					Repro	2,133			
Smith et al. 1988									**4-nitrophenol**
2	Rat (Crl:CD) 10 M	2 weeks 5 days/week 6 hours/day (H)	0, 26, 112	LE, CS, BW, BC, HE, UR, GN, OW, HP	Resp	112			
					Cardio	112			
					Gastro	112			
					Hemato	26	112		Methemoglobin increased from 0.5% (in controls) to 1.5%
					Hepatic	112			
					Renal	112			
					Dermal	112			
					Ocular	112			
					Endocr	112			
					Immuno	112			
					Neuro	112			
					Repro	112			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Smith et al. 1988									**4-nitrophenol**
3	Rat (Crl:CD) 6 M	4 hours (H)	1,304, 4,059	CS	Ocular	1,304		4,059	Corneal opacity
INTERMEDIATE EXPOSURE									
Hazleton 1983									**4-nitrophenol**
4	Rat (Sprague-Dawley) 15 M,15 F	4 weeks 5 days/week 6 hours/day (WB)	0, 1.09, 5.27, 29.18	LE, CS, BW, BC, HE, OP, GN, OW, HP	Bd wt	29.18			
					Resp	29.18			
					Cardio	29.18			
					Gastro	29.18			
					Hemato	29.18			
					Musc/skel	29.18			
					Hepatic	29.18			
					Renal	29.18			
					Dermal	29.18			
					Ocular	5.27 M 29.18 F		29.18 M	Cataracts
					Endocr	29.18			
					Immuno	29.18			
					Neuro	29.18			
					Repro	29.18			
Hazleton 1984									**2-nitrophenol**
5	Rat (Sprague-Dawley) 15 M, 15 F	4 weeks 5 days/week 6 hours/day (WB)	0, 5.0, 32.5, 61.5	LE, CS, BW, BC, HE, OP, GN, OW, HP	Bd wt	61.5			
					Resp	5.0	32.5		Squamous metaplasia of the epithelium lining the nasal turbinates
					Cardio	61.5			
					Gastro	61.5			
					Hemato	61.5			
					Musc/skel	61.5			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic	61.5			
					Renal	61.5			
					Dermal	61.5			
					Ocular	61.5			
					Endocr	61.5			
					Immuno	61.5			
					Neuro	61.5			
					Repro	61.5			

[a]The number corresponds to entries in Figure 2-2; differences in levels of health 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.

BC = blood chemistry; Bd wt or BW = body weight; Cardio = cardiovascular; CS = clinical signs; Endocr = endocrine; F = female(s); Gastro = gastrointestinal; GN = gross necropsy; (H) = head-only; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = musculoskeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; OP = ophthalmology; OW = organ weight; Repro = reproductive; Resp = respiratory; UR = urinalysis; (WB) = whole-body

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

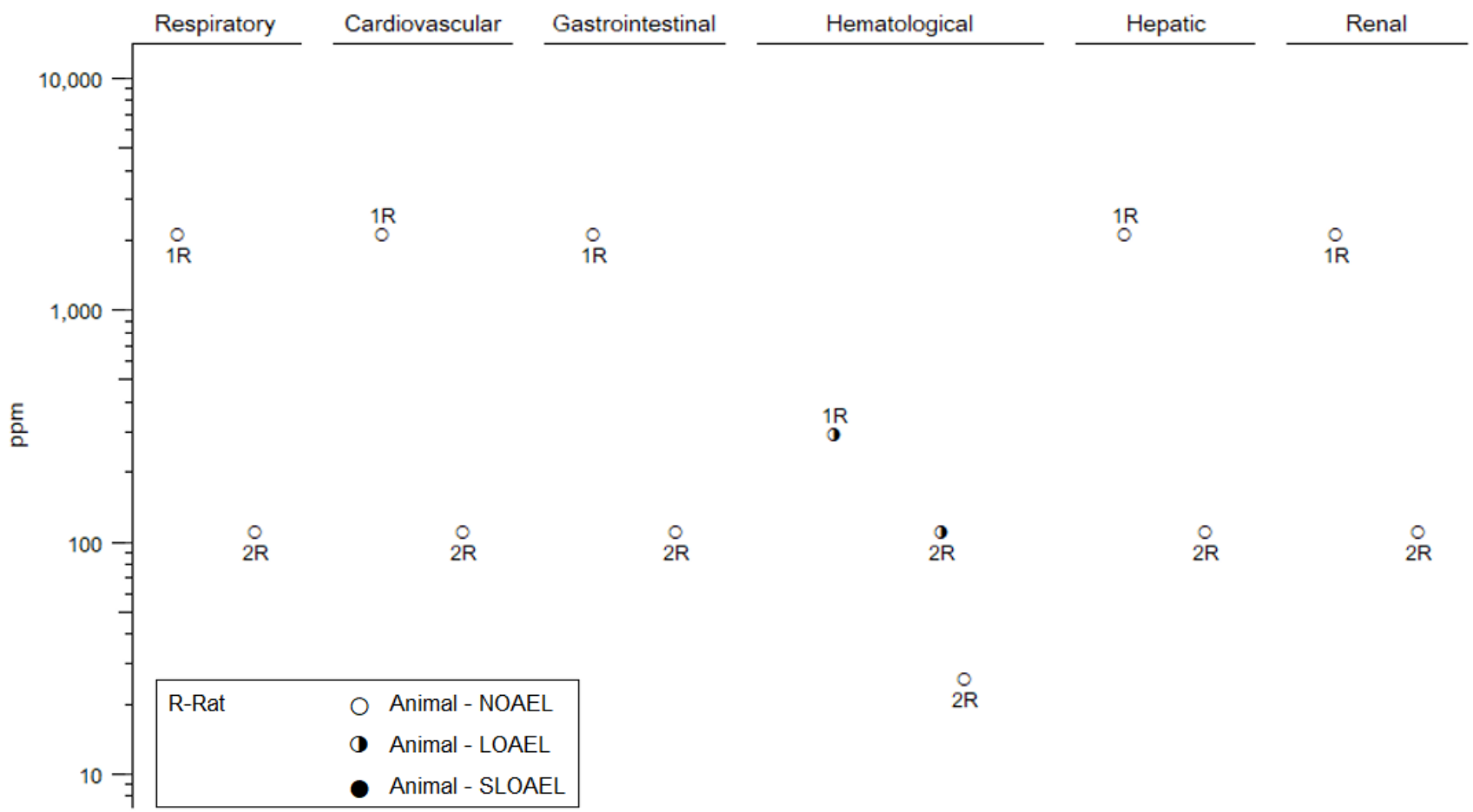


Figure 2-2. Levels of Significant Exposure to Nitrophenols – Inhalation
Acute (≤14 days)

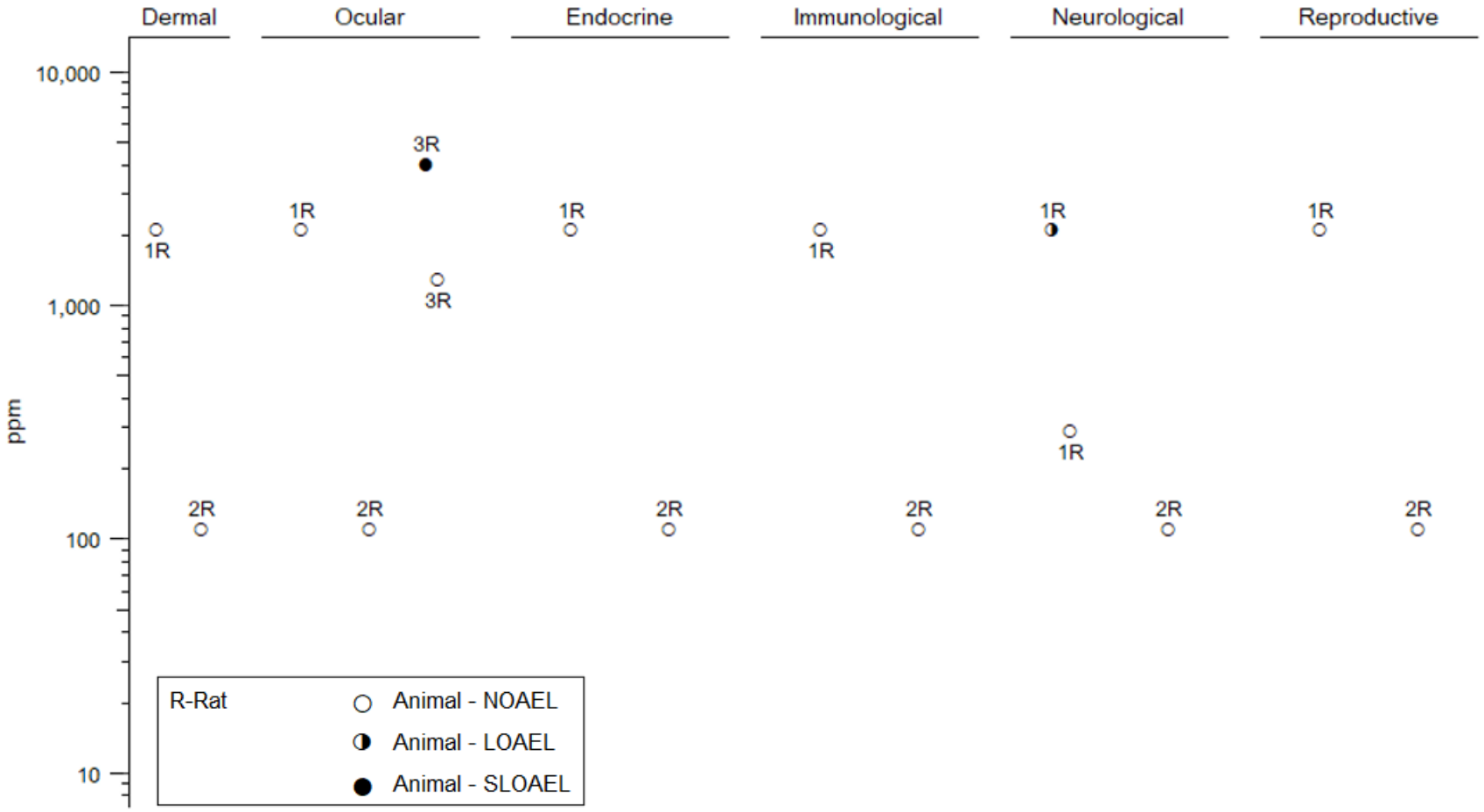


Figure 2-2. Levels of Significant Exposure to Nitrophenols – Inhalation
Intermediate (15-364 days)

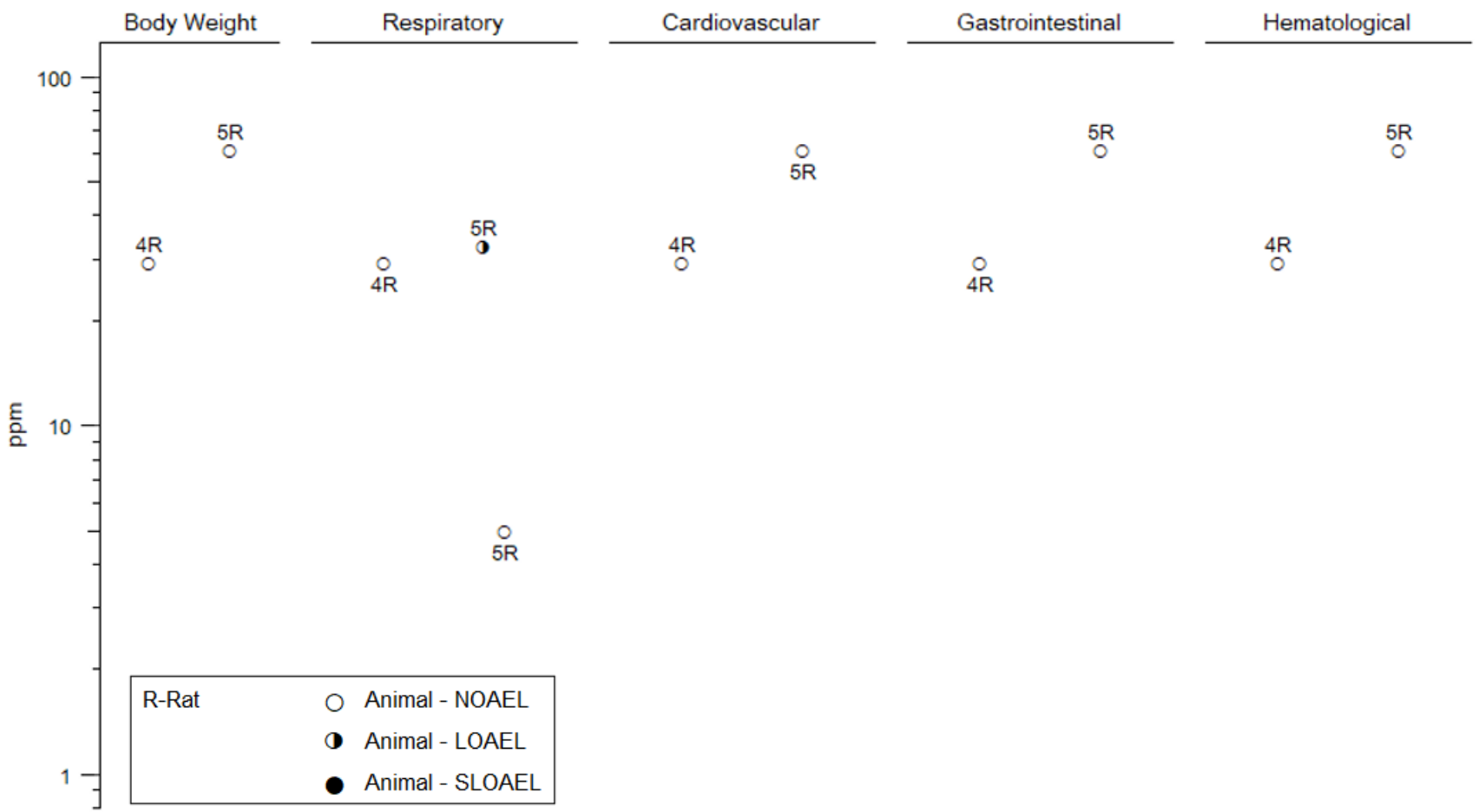


Figure 2-2. Levels of Significant Exposure to Nitrophenols – Inhalation
Intermediate (15-364 days)

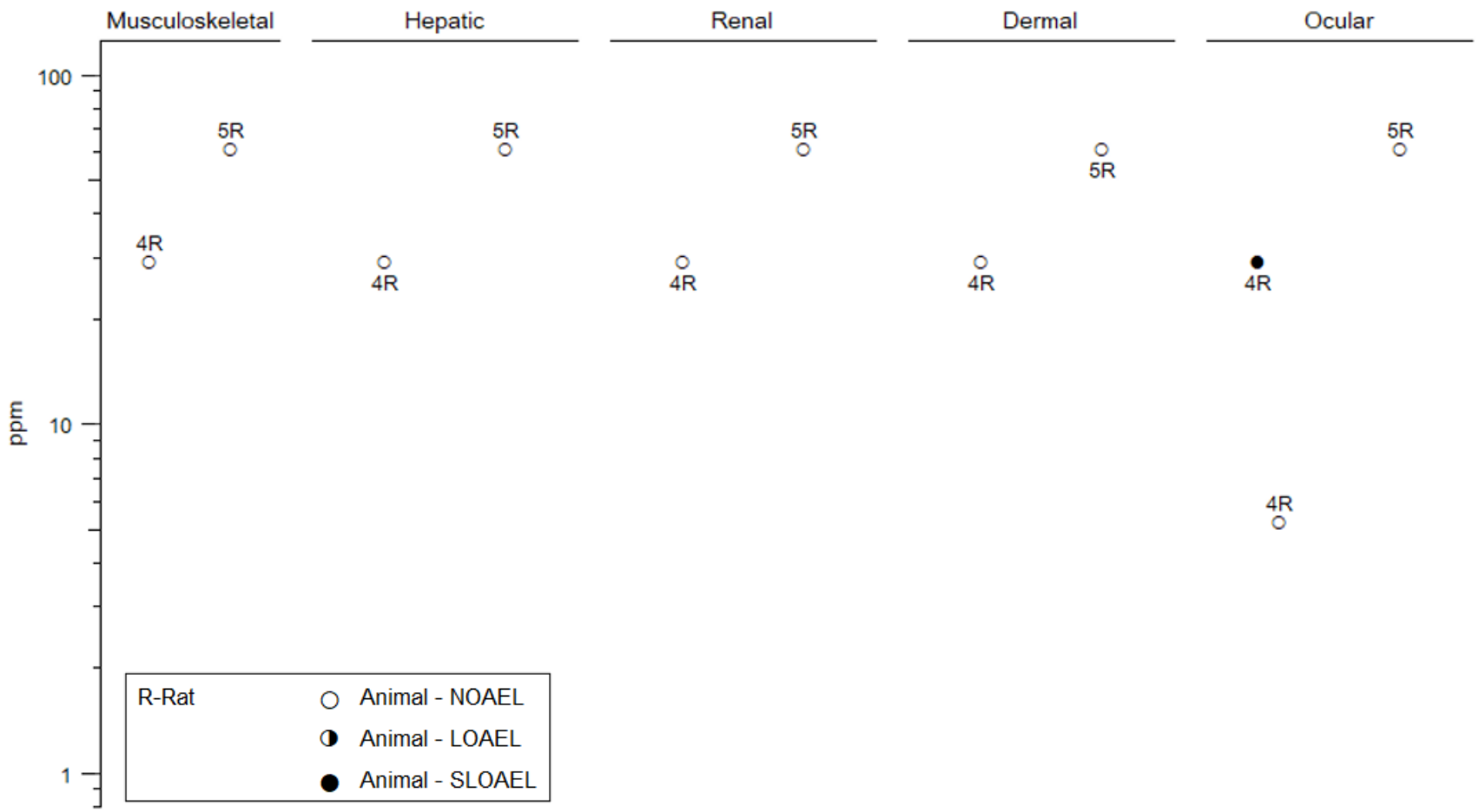


Figure 2-2. Levels of Significant Exposure to Nitrophenols – Inhalation
Intermediate (15-364 days)

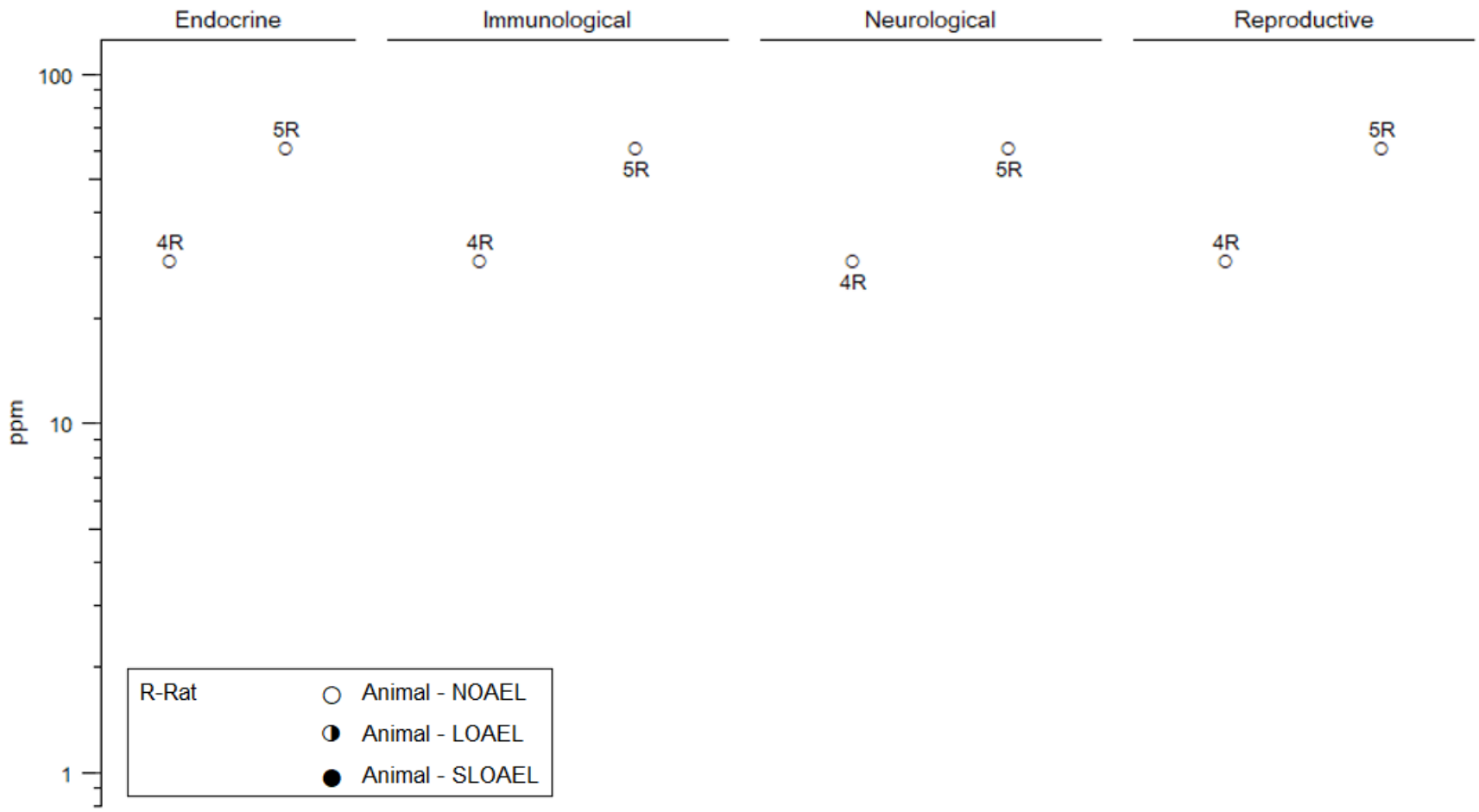


Table 2-2. Levels of Significant Exposure to Nitrophenols – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Abu-Qare et al. 2000									**4-nitrophenol**
1	Rat (Sprague-Dawley) 7–21 F	Once GD 14, 15, 16, 17, or 18 (GW)	0, 100	BW, FI, WI, HE, BI, OW, DX	Bd wt	100			
					Hemato	100			
					Neuro	100			
					Repro	100			
					Develop	100			
Branch et al. 1983b									**4-nitrophenol**
2	Rat (Albino) 25 M, 25 F	Once (G)	70, 110, 171, 268, 420	LE, CS	Death			171 F 268 M	3/5 females died at 171 mg/kg; 4/5 males died at 268 mg/kg (LD_{50} = 230 mg/kg/)
					Resp	70 F 171 M		171 F 268 M	Dyspnea prior to death
					Neuro	110		268	Convulsions and prostration prior to death
EPA 1992a									**4-nitrophenol**
3	Rat (Sprague-Dawley) 20 F	10 days GDs 6–16 (G)	0, 1.4, 13.8, 27.6	LE, BW, GN, RX, DX	Bd wt	13.8	27.6		12% decrease in maternal body weight
					Repro	27.6			
					Develop	27.6			
Kavlock 1990									**4-nitrophenol**
4	Rat (Sprague-Dawley) 12 F	Once GD 11 (G)	0, 333, 667, 1,000	LE, CS, BW, DX	Death			667	3/13 died
					Bd wt	1,000			
					Develop	1,000			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Koizumi et al. 2001									**4-nitrophenol**
5	Rat (Sprague-Dawley) 5 M, 5 F	14 days (G)	0, 3, 12.5, 50, 200	LE, CS, BW, HE	Bd wt	200			
					Hemato	200			
Laughlin et al. 1983									**2- nitrophenol**
6	Rat (Sprague-Dawley) 5 F	10 days GDs 6–15 (GO)	0, 50, 125, 250, 500, 1,000	LE, CS, BW, RX	Bd wt	1,000			
					Repro	500		1,000	Post implantation loss
Li et al. 2017; Tang et al. 2016									**4-nitrophenol**
7	Rat (Wistar) 9 M	Once (G)	0, 200	BW, BI, OW, HP	Bd wt	200			
					Gastro		200		Necrosis and desquamation of the mucosal epithelium of small intestine; loss of goblet cells
					Hepatic		200		Disordered hepatocytes; detached central vein of the hepatic lobule
					Renal	200			
					Endocr	200			
					Immuno	200			
					Repro	200			
[Histology evaluated in liver and small intestine only.]									
Li et al. 2017; Tang et al. 2016									**4-nitrophenol**
8	Rat (Wistar) 12 M	3 days (G)	0, 200	BW, BI, OW, HP	Bd wt			200	25% decrease in body weight
					Gastro		200		Necrosis and desquamation of the mucosal epithelium of small intestine; loss of goblet cells
					Hepatic		200		Disordered hepatocytes, widened hepatic sinusoid

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Renal	200			
					Endocr	200			
					Immuno	200			
					Repro	200			
[Histology evaluated in liver and small intestine only.]									
Vernot et al. 1977									**4- nitrophenol**
9	Rat (Sprague-Dawley) NS M	Once (NS)	Not reported	LE	Death			620	LD_{50}
Vernot et al. 1977									**2- nitrophenol**
10	Rat (Sprague-Dawley) NS M	Once (NS)	Not reported	LE	Death			2,830	LD_{50}
Vernot et al. 1977									**3-nitrophenol**
11	Rat (Sprague-Dawley) NS M	Once (NS)	Not reported	LE	Death			930	LD_{50}
Plasterer et al. 1985									**4-nitrophenol**
12	Mouse (CD1) 10 F	8 days GDs 7–14 (GO)	0, 400	LE, CS, BW CS, RX, DX	Death			400	19% decrease in maternal survival
					Bd wt		400		18% decrease in maternal body weight gain
					Repro	400			
					Develop	400			
Plasterer et al. 1985									**4-nitrophenol**
13	Mouse (CD1) 10 F	8 days (GO)	0, 62.5, 125, 250, 500, 1,000	LE, BW	Death			625.7	LD_{50}
					Bd wt	1,000			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Vernot et al. 1977									**2-nitrophenol**
14	Mouse (CF-1) NS	Once (NS)	Not reported	LE	Death			1,300	LD_{50}
Vernot et al. 1977									**4-nitrophenol**
15	Mouse (CF-1) NS	Once (NS)	Not reported	LE	Death			470	LD_{50}
Vernot et al. 1977									**3-nitrophenol**
16	Mouse (CF-1) NS	Once (NS)	Not reported	LE	Death			1,410	LD_{50}
INTERMEDIATE EXPOSURE									
Hazleton 1989									**4-nitrophenol**
17	Rat (Sprague-Dawley) 20 M, 20 F	13 weeks 7 days/week (GW)	0, 25, 70, 140	LE, CS, FI, BW, BC, OP, OW, GN, HP	Death			140	15/20 males and 6/20 females died
					Bd wt	140			
					Resp	70		140	Dyspnea and wheezing (prior to death)
					Cardio	140			
					Gastro	140			
					Hemato	140			
					Musc/skel	140			
					Hepatic	140			
					Renal	140			
					Dermal	140			
					Ocular	140			
					Endocr	140			
					Immuno	140			
					Neuro	70		140	Prostration (prior to death)
					Repro	140			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Koizumi et al. 2001									**4-nitrophenol**
18	Rat (Sprague-Dawley) 12 M, 12 F	18 days PNDs 4–21 (G)	0, 80, 110, 160	DX	Develop	160			
Koizumi et al. 2001									**4-nitrophenol**
19	Rat (Sprague-Dawley) 6 M, 6 F	18 days PNDs 4–21 (G)	0, 110, 160, 230, 320	LE, CS	Death			320 F	6/6 females died
								230 M	3/6 males died
					Neuro	160		230	Convulsions
Koizumi et al. 2001									**4-nitrophenol**
20	Rat (Sprague-Dawley) 12 M, 12 F	28 days (G)	0, 60, 160, 400, 1,000	LE, CS, BW, FI, BC, HE, UR, GN, OW, HP	Death			1,000	10/12 males and 10/12 females died
					Bd wt	1,000			
					Resp	400		1,000	Oligopnea
					Cardio	1,000			
					Gastro	1,000			
					Hepatic	1,000			
					Renal	160	400		Eosinophilic bodies in proximal renal tubular cells
					Endocr	1,000			
					Immuno	1,000			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Neuro	400		1,000	Tonic convulsions, decreased locomotor activity, prostration
					Repro	1,000			

[a]The number corresponds to entries in Figure 2-3; differences in levels of health 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.

BC = blood chemistry; Bd wt or BW = body weight; BI = biochemical indices; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; F = female(s); FI = food intake; (G) = gavage, neat or vehicle other than water or oil; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; (GO) = gavage with oil vehicle; (GW) = gavage with water vehicle; 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/skel = musculoskeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OP = ophthalmology; OW = organ weight; PND = postnatal day; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; UR = urinalysis; WI = water intake

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

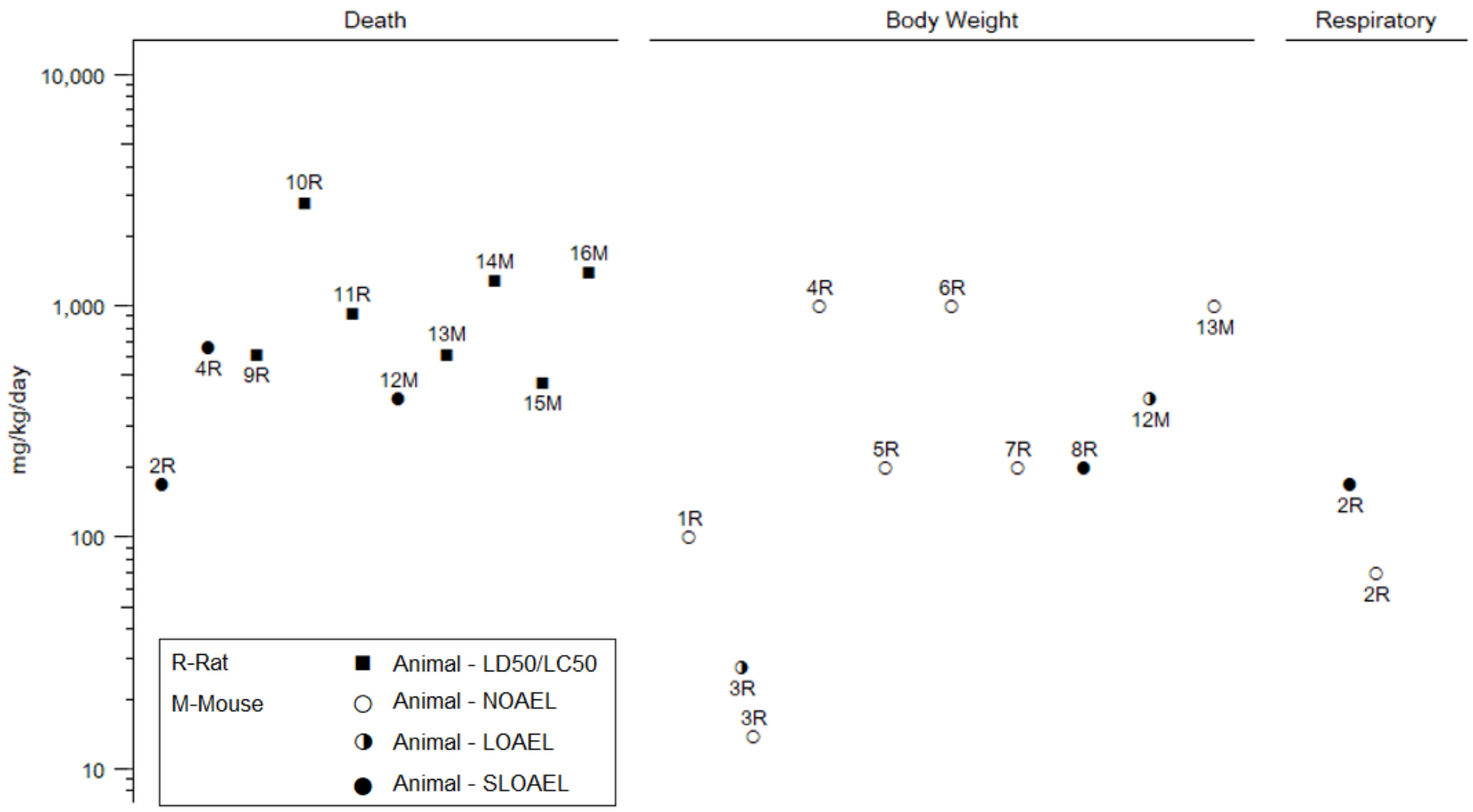


Figure 2-3. Levels of Significant Exposure to Nitrophenols – Oral
Acute (≤14 days)

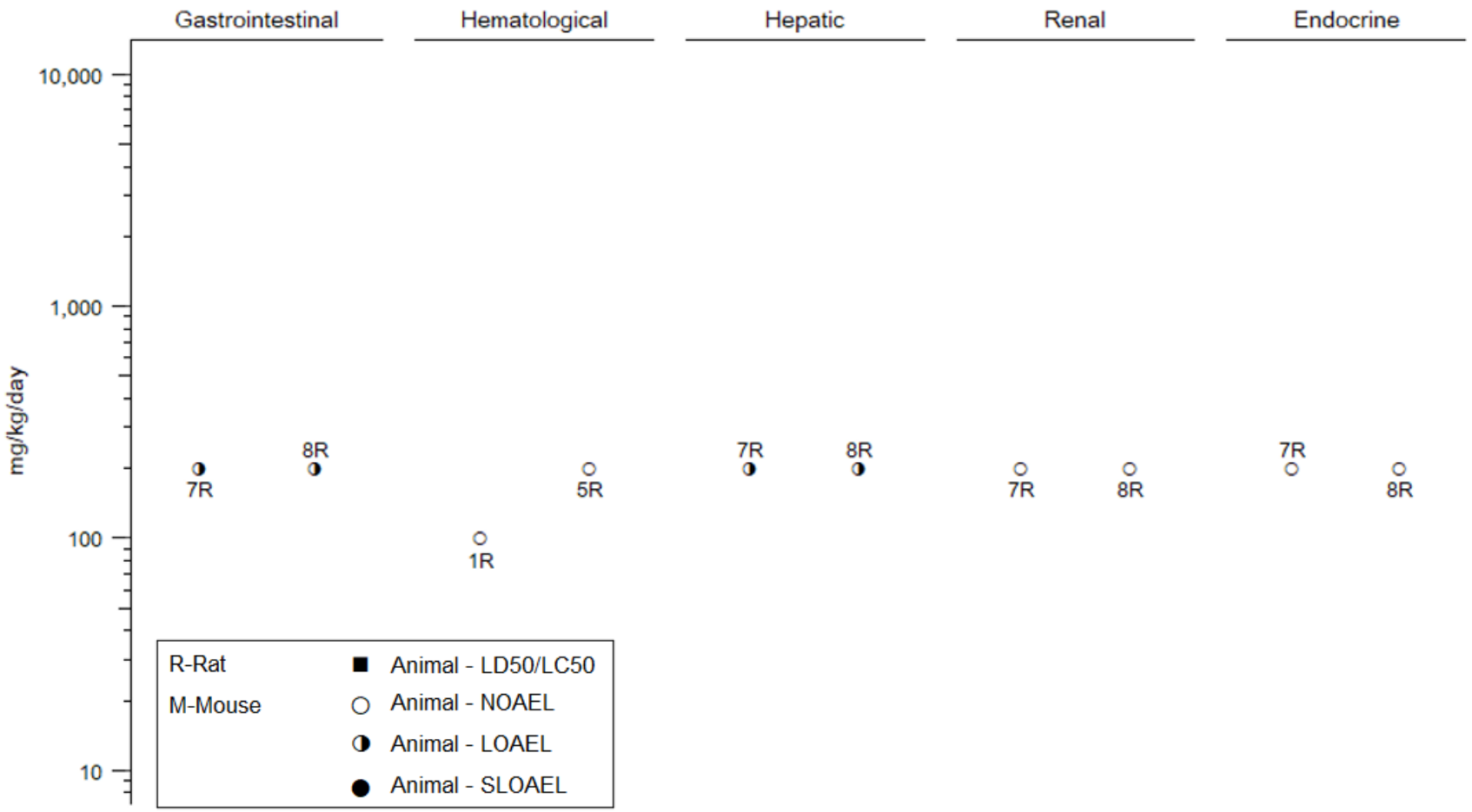


Figure 2-3. Levels of Significant Exposure to Nitrophenols – Oral
Acute (≤14 days)

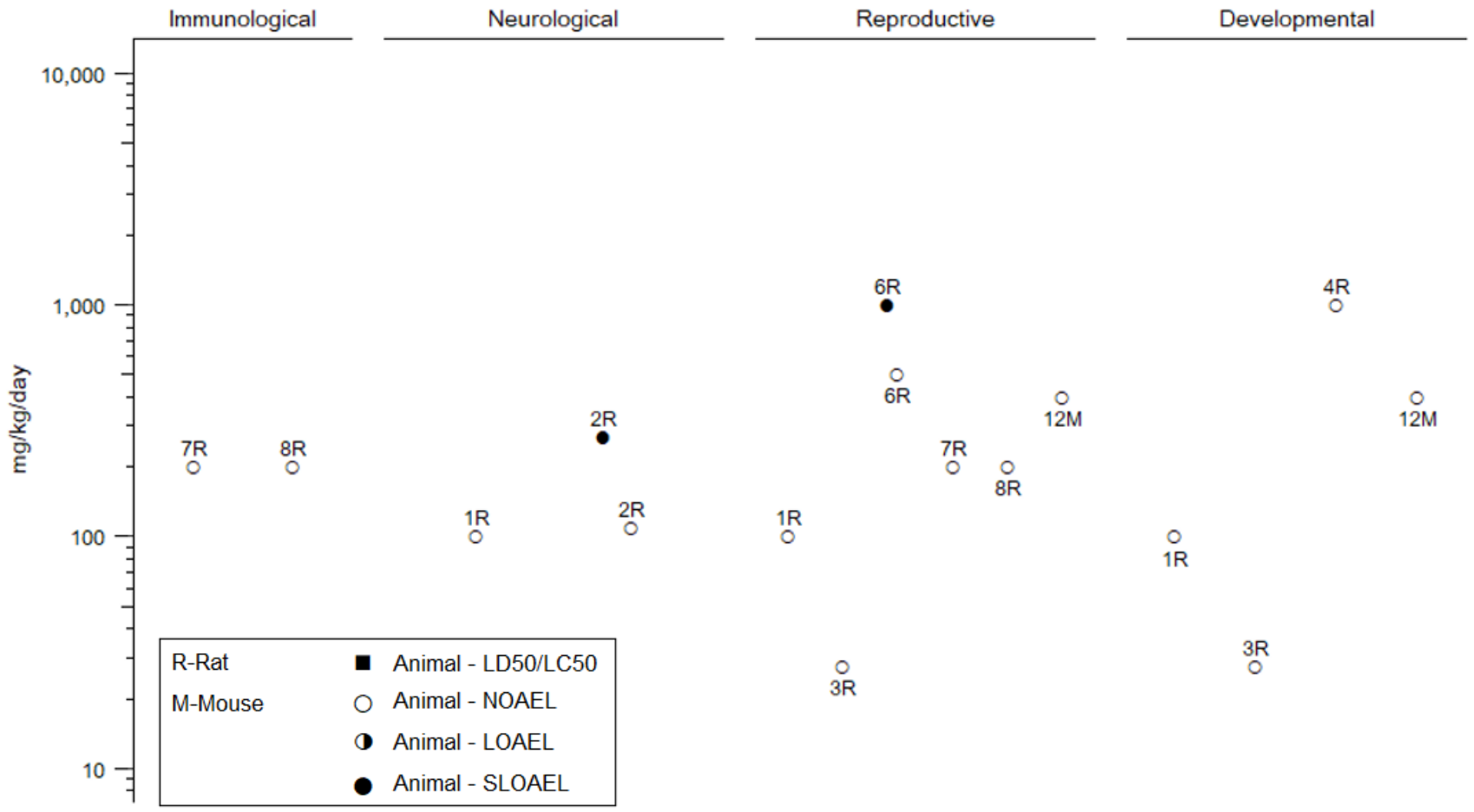


Figure 2-3. Levels of Significant Exposure to Nitrophenols – Oral
Intermediate (15-364 days)

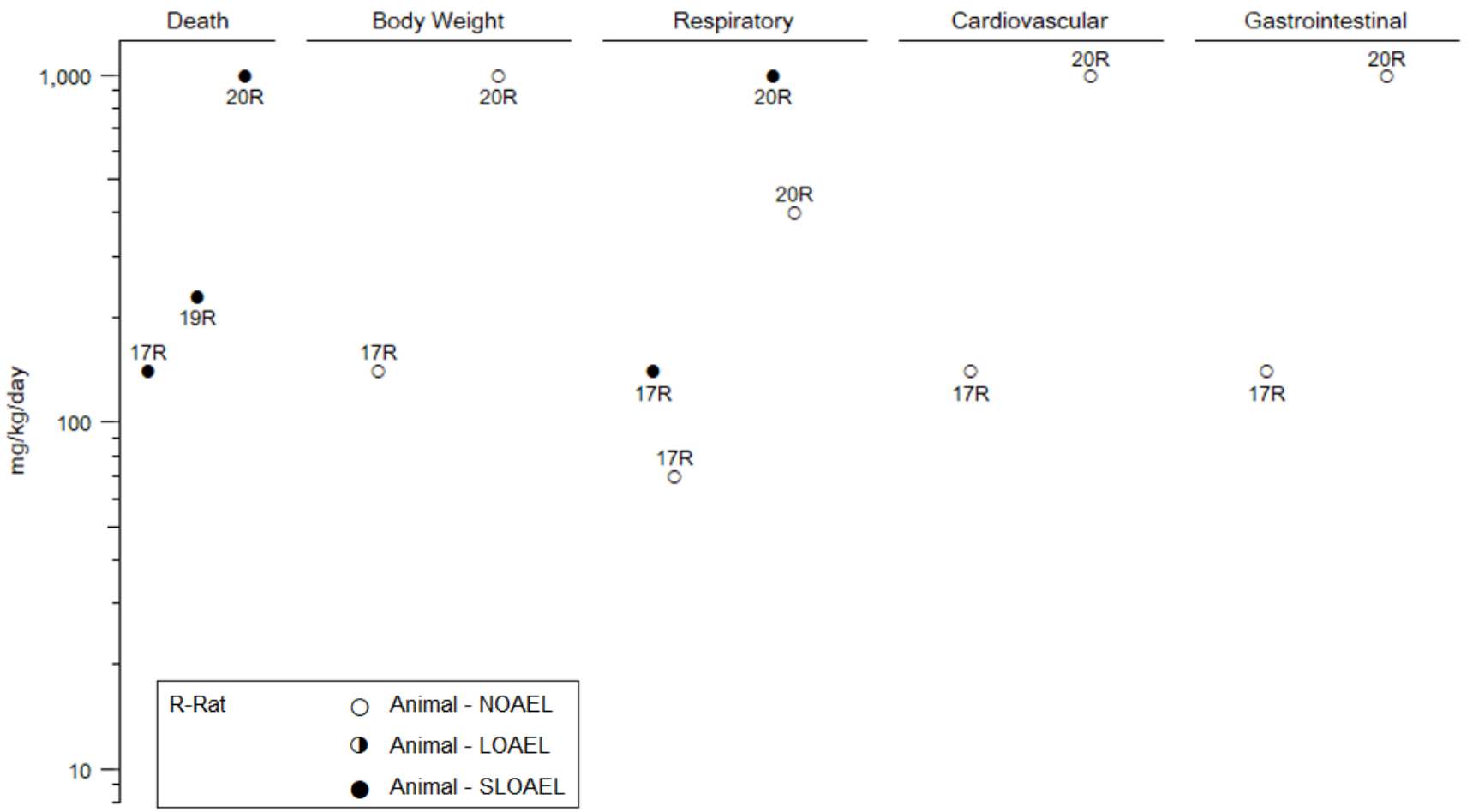


Figure 2-3. Levels of Significant Exposure to Nitrophenols – Oral
Intermediate (15-364 days)

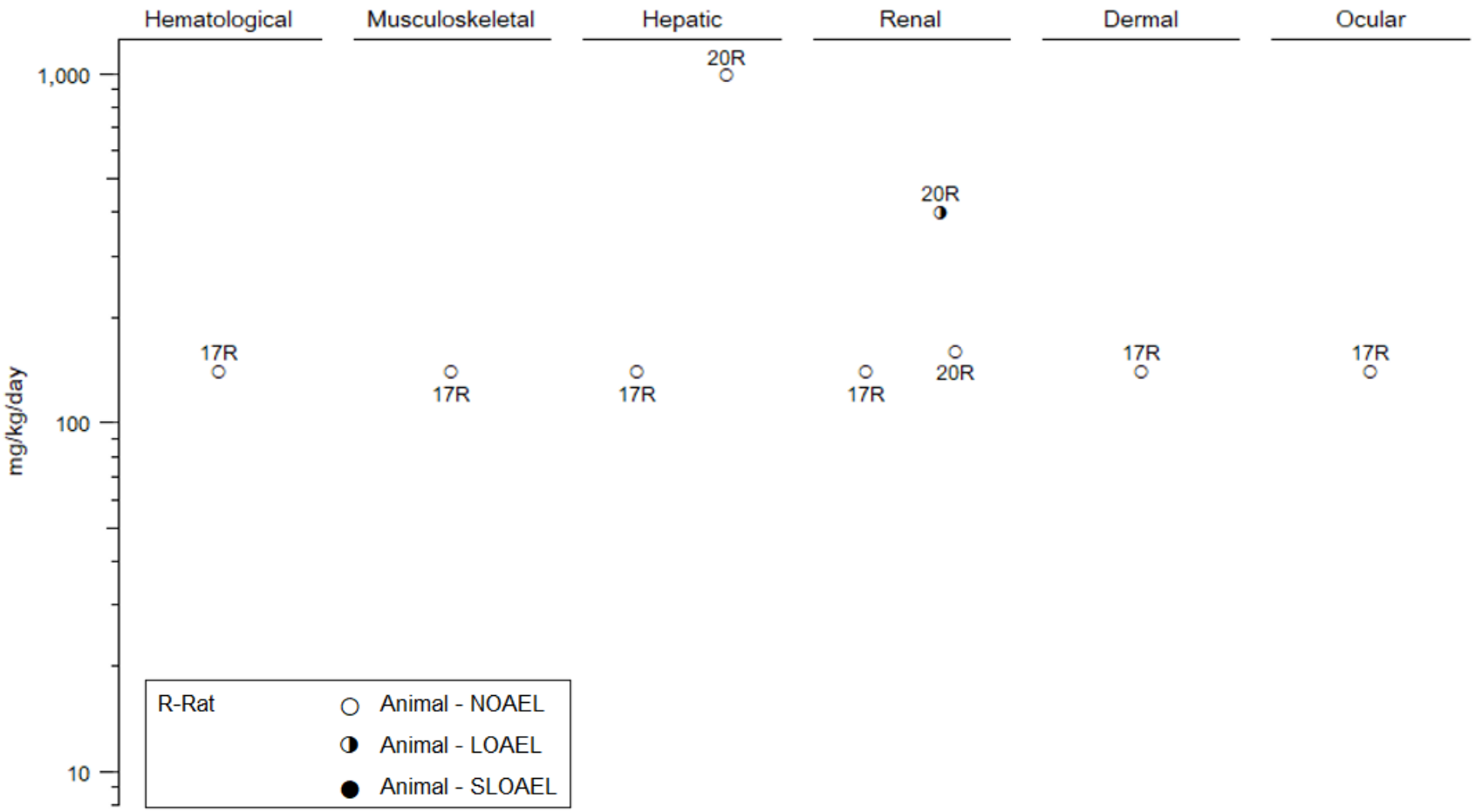


Figure 2-3. Levels of Significant Exposure to Nitrophenols – Oral
Intermediate (15-364 days)

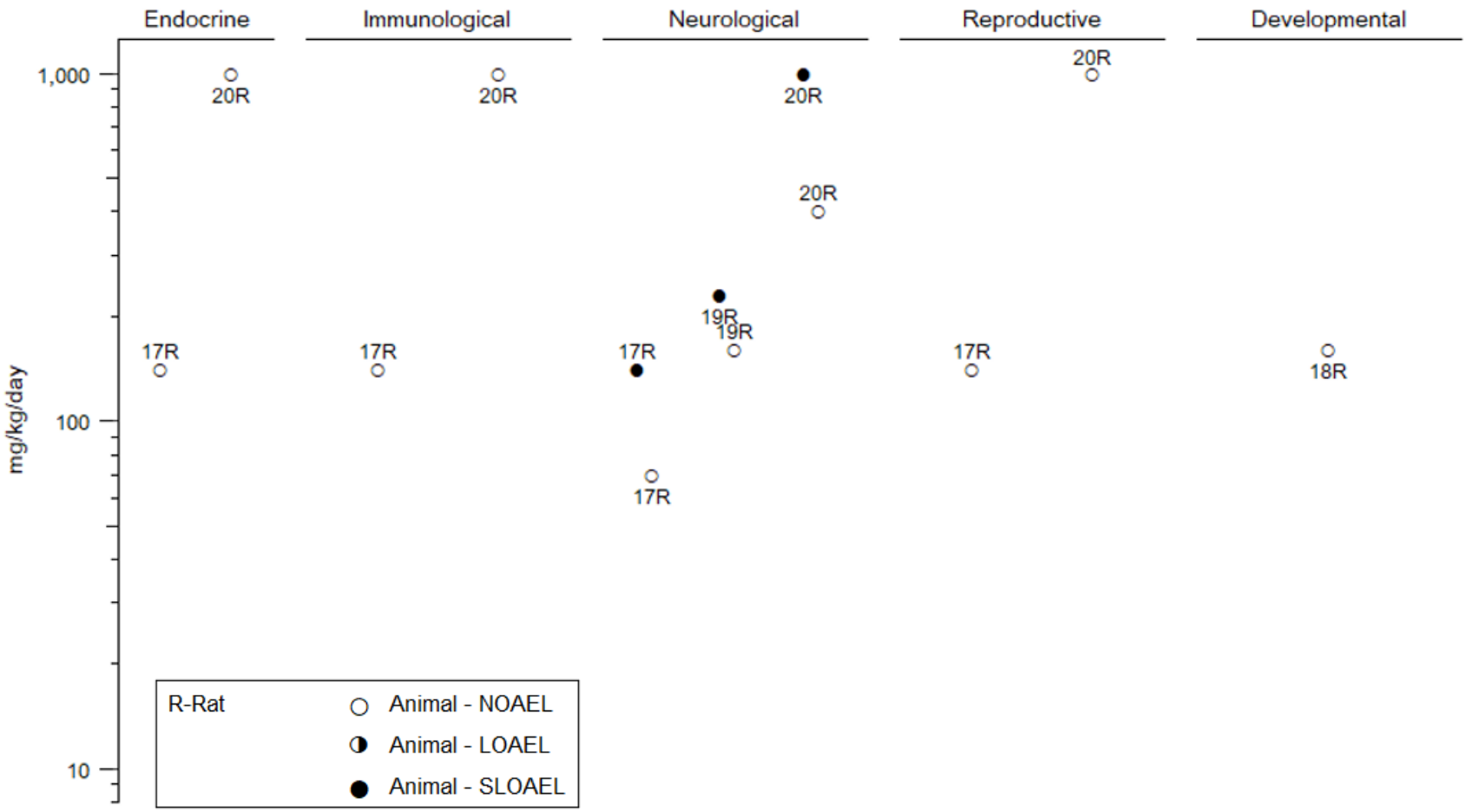


Table 2-3. Levels of Significant Exposure to Nitrophenols – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE								
Branch et al. 1983a								**4-nitrophenol**
Rabbit (New Zealand white) 5 M, 5 F	24 hours (occluded conditions)	5,000 mg/kg in saline	LE, CS, GN	Dermal		5,000 mg/kg		Erythema and edema
EPA 1992a								**4-nitrophenol**
Rabbit (New Zealand white) 6 NS	Once (conjunctival sac)	100 mg (solid)	CS	Ocular			100 mg	Severe irritation and corneal opacity; inflammation and visible destruction of iris
Monsanto 1983a								**4-nitrophenol**
Rabbit (New Zealand white) 3 M, 3 F	Once (conjunctival sac)	70 mg (solid)	CS	Ocular			70 mg	Corneal cloudiness, corneal neovascularization, blistering of conjunctival tissue
Monsanto 1983b								**4-nitrophenol**
Rabbit (New Zealand white) 3 M, 3 F	24 hours (occluded conditions)	184 mg/kg in saline	CS, GN	Dermal		184 mg/kg		Skin scabbing and scarring
Monsanto 1984								**4-nitrophenol**
Rabbit (New Zealand white) 3 M, 3 F	4 hours (occluded conditions)	0, 148 mg/kg in saline	CS, GN	Dermal		148 mg/kg		Erythema and edema
INTERMEDIATE EXPOSURE								
U.S. Army 1985								**4-nitrophenol**
Rat (Sprague-Dawley) F0: 12 M, 24 F F1: 13 M, 26 F	2 generations 20–24 weeks per generation 5 days/week	0, 50, 100, 250 mg/kg/day in ethanol	LE, CS, BW, OW, HP, RX, DX	Bd wt	250 mg/kg/day			
				Cardio	250 mg/kg/day			
				Hepatic	250 mg/kg/day			
				Renal	250 mg/kg/day			

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

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
				Dermal		50 mg/kg/day		Skin irritation (erythema, scaling, scabbing, cracking) and histopathological changes in the skin (chronic inflammation, acanthosis, eschar, sebaceous hypertrophy) in F0 and F1 rats
				Neuro	250 mg/kg/day			
				Repro	250 mg/kg/day			
				Develop	250 mg/kg/day			
CHRONIC EXPOSURE								
NTP 1993								**4-nitrophenol**
Mouse (Swiss-Webster) 60 M, 60 F	78 weeks 3 days/week	0, 40, 80, 160 mg/kg/day in acetone	LE, CS, BW, GN, HP	Bd wt	160 mg/kg/day			
				Resp	160 mg/kg/day			
				Cardio	160 mg/kg/day			
				Gastro	160 mg/kg/day			
				Musc/skel	160 mg/kg/day			
				Hepatic	160 mg/kg/day			
				Renal	160 mg/kg/day			
				Dermal	160 mg/kg/day			
				Ocular	160 mg/kg/day			
				Endocr	160 mg/kg/day			
				Immuno	160 mg/kg/day			
				Neuro	160 mg/kg/day			
				Repro	160 mg/kg/day			

Bd wt or BW = body weight; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; F = female(s); Gastro = gastrointestinal; GN = gross necropsy; HP = histopathology; Immuno = immunological; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = musculoskeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OW = organ weight; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity

2.2 DEATH

No studies were identified regarding death in humans after exposure to 2-, 3-, or 4-nitrophenol. In animals, mortality was evaluated following inhalation exposure to 2- or 4-nitrophenol; oral exposure to 2-, 3-, or 4-nitrophenol; and dermal exposure to 2- or 4-nitrophenol.

No exposure-related mortalities were observed in rats exposed to 4-nitrophenol following exposure to concentrations up to 4,059 mg/m^3 for 4 hours (Smith et al. 1988) or intermittent exposure to concentrations up to 2,133 mg/m^3 for 2 weeks (Smith et al. 1988) or 29.18 mg/m^3 for 4 weeks (Hazleton 1983). Similarly, no exposure-related deaths were observed in rats intermittently exposed to 2-nitrophenol concentrations up to 61.5 mg/m^3 for 4 weeks (Hazleton 1984).

In acute oral lethality studies, reported oral median lethal dose (LD_{50}) values were 2,830 mg/kg for rats and 1,300 mg/kg for mice exposed to 2-nitrophenol (Vernot et al. 1977), 930 mg/kg for rats and 1,410 mg/kg for mice exposed to 3-nitrophenol (Vernot et al. 1977), and 202–620 mg/kg for rats and 470–625.7 mg/kg for mice exposed to 4-nitrophenol (Branch et al. 1983b; Plasterer et al. 1985; Vernot et al. 1977). The cause of death was not reported in acute lethality studies; however, Branch et al. (1983b) reported dyspnea, convulsions, and/or prostration prior to death. In a 14-day gavage study, no exposure-related deaths were reported in rats exposed to 4-nitrophenol doses up to 200 mg/kg/day for 14 days (Koizumi et al. 2001).

In pregnant rodents, 3/13 maternal rats died following a single exposure to 667 mg/kg on gestational day (GD) 11 (Kavlock 1990) and a 19% decreased in maternal survival was observed in mice exposed to 400 mg/kg/day on GDs 7–14 (Plasterer et al. 1985). No maternal deaths were reported in rats exposed to 4-nitrophenol at doses up to 27.6 mg/kg/day on GDs 6–16 (EPA 1992a) or 2-nitrophenol at doses up to 1,000 mg/kg/day on GDs 6–15 (Laughlin et al. 1983).

Increased mortality was also reported in rats following intermediate-duration oral exposure to 4-nitrophenol. In adult rats, 10/12 males and 10/12 females died following gavage exposure to 1,000 mg/kg/day for up to 28 days (Koizumi et al. 2001) and 15/20 males and 6/20 females died following gavage exposure to 140 mg/kg/day for up to 13 weeks (Hazleton 1989). Clinical signs observed prior to death included slow, shallow, or irregular breathing and neurological effects (convulsions, decreased activity, and/or prostration). In neonatal mice exposed to 4-nitrophenol on postnatal days (PNDs) 4–21 via gavage, decreased survival was observed at 230 mg/kg/day in male rat

pups (3/6 died) and 320 mg/kg/day in female rat pups (6/6 died); convulsions were observed prior to death (Koizumi et al. 2001).

No exposure-related mortalities were observed in animals following dermal exposure to nitrophenols. In an acute dermal lethality study, all rabbits exposed to 4-nitrophenol at a dose of 5,000 mg/kg in saline under occluded conditions for 24 hours survived (Branch et al. 1983a). In dermal studies in mice, decreased survival was not observed following exposure to 2- or 4-nitrophenol diluted in dioxane to a dose of 138 mg/kg/day applied twice weekly for 12 weeks (Boutwell and Bosch 1959) or doses up to 160 mg/kg/day diluted in acetone 3 days/week for 78 weeks (NTP 1993). Similarly, no exposure-related deaths were observed in F0 or F1 rats exposed to 4-nitrophenol doses up to 250 mg/kg/day diluted in ethanol over two generations (5 days/week; 20–24 weeks per generation) (U.S. Army 1985).

2.3 BODY WEIGHT

No studies were identified regarding body weight in humans after exposure to 2-, 3-, or 4-nitrophenol. In animals, body weights were evaluated following inhalation exposure to 2- or 4-nitrophenol, oral exposure to 2- or 4-nitrophenol, and dermal exposure to 4-nitrophenol. Based on a systematic evaluation of the literature, body weight effects are not classifiable as a health effect of oral exposure to 4-nitrophenol. The full results of the systematic review are presented in Appendix C.

Decreased body weight gains of unspecified magnitudes were reported in rats following exposure to 4-nitrophenol at concentrations ≥1,304 mg/m^3 for 4 hours or intermittent exposure to ≥294 mg/m^3 for 2 weeks (Smith et al. 1988). In a second 2-week inhalation study with 4-nitrophenol, Smith et al. (1988) observed lower than expected body weight gains in both control and exposed rats (up to 112 mg/m^3). The study authors attributed the low body weight gain to stress associated with handling. Due to lack of quantitative data reported as well as potential stress-related body weight effects, NOAEL and LOAEL calls for body weight could not be determined for this study. In intermediate-duration inhalation studies, no exposure-related changes in body weight were observed in rats intermittently exposed to 2-nitrophenol at concentrations up to 61.5 mg/m^3 (Hazleton 1984) or 4-nitrophenol at concentrations up to 29.18 mg/m^3 (Hazleton 1983).

Evidence for body weight effects following acute-duration oral exposure to nitrophenols is mixed. No body weight effects were noted in rats exposed once to 4-nitrophenol via gavage at doses up to 1,000 mg/kg (Abu-Qare et al. 2000; Kavlock 1990; Li et al. 2017; Tang et al. 2016). One study reported

a transient 25% decrease in body weights of male rats exposed to 200 mg/kg/day of 4-nitrophenol via gavage for 3 days (body weights were comparable to control after a 3-day recovery period) (Li et al. 2017; Tang et al. 2016). However, Koizumi et al. (2001) did not observe exposure-related changes in body weights in male or female rats exposed to 200 mg/kg/day of 4-nitrophenol via gavage for 14 days. In pregnant rats, a 12% decrease in maternal body weights was observed in dams exposed to 27.6 mg/kg/day of 4-nitrophenol via gavage on GDs 6–16 (EPA 1992a). However, no exposure-related changes in maternal body weights were observed in rat dams exposed to 2-nitrophenol via gavage at doses up to 1,000 mg/kg/day on GDs 6–15. In mice, an 18% decrease in maternal body weight gain was observed following exposure to 400 mg/kg/day of 4-nitrophenol via gavage for 8 days on GDs 7–14; however, no exposure-related changes in body weight were observed in nonpregnant mice similarly exposed for 8 days to doses up to 1,000 mg/kg/day (Plasterer et al. 1985).

Intermediate-duration exposure to 4-nitrophenol showed no body weight effects in rats following gavage exposure to doses up to 1,000 mg/kg/day for 28 days (Koizumi et al. 2001) or 140 mg/kg/day for 13 weeks (Hazleton 1989).

In a 2-generation dermal study in rats, no body weight effects were reported in F0 or F1 rats exposed intermittently to 4-nitrophenol doses up to 250 mg/kg/day for 20–24 weeks per generation (U.S. Army 1985). Similarly, no body weight effects were observed in Swiss-Webster mice exposed intermittently to dermal applications of 4-nitrophenol at doses up to 160 mg/kg/day for 78 weeks (NTP 1993).

2.4 RESPIRATORY

No studies were identified regarding respiratory effects in humans after exposure to 2-, 3-, or 4-nitrophenol. In animals, respiratory toxicity was evaluated in a single inhalation study of exposure to 2-nitrophenol and inhalation, oral, and dermal studies of exposure to 4-nitrophenol. The upper respiratory tract (nasal tissue) appears to be a sensitive target of inhalation exposure to 2-nitrophenol in rats; however, since data are limited to a single study, a systematic review could not be conducted for respiratory effects following inhalation exposure to 2-nitrophenol. Respiratory effects were only noted following 4-nitrophenol exposure to oral doses associated with mortality.

In the only inhalation study evaluating 2-nitrophenol, rats intermittently exposed for 4 weeks showed squamous metaplasia of the nasal epithelium of 10/10 males and 9/10 females exposed to 32.5 mg/m^3 and 10/10 males and 10/10 females at 61.5 mg/m^3 (Hazleton 1984). Incidence of this lesions was 1/sex in

control animals and 0/10 males and 1/10 females at 5 mg/m^3. No exposure-related changes in lung weight or histology were observed at concentrations up to 61.4 mg/m^3, and no clinical signs of respiratory distress were noted.

In inhalation studies evaluating 4-nitrophenol, no clinical signs of respiratory distress or adverse changes in lung weight or histology were observed in rats intermittently exposed to concentrations up to 2,133 mg/m^3 for 2 weeks (Smith et al. 1988) or 29.18 mg/m^3 for 4 weeks (Hazleton 1983). Additionally, no exposure-related lesions were observed in nasal tissues of rats intermittently exposed to 29.18 mg/m^3 for 4 weeks (Hazleton 1983).

As discussed in Section 2.2 (Death), clinical signs of respiratory distress were often reported prior to death following exposure to lethal oral doses of 4-nitrophenol. Dyspnea was reported in rats exposed once to gavage doses ≥171 mg/kg (Branch et al. 1983b). In intermediate-duration gavage studies, Koizumi et al. (2001) reported oligpnea (shallow or slow breathing) in rats exposed to 1,000 mg/kg/day for up to 28 days and Hazleton (1989) reported dyspnea and wheezing prior to death in rats exposed to 140 mg/kg/day for up to 13 weeks. No clinical signs of respiratory distress were noted at nonlethal doses, and no exposure related changes in lung weight or histology were noted in the intermediate-duration gavage studies (not evaluated in the acute-duration gavage study).

In a chronic-duration dermal study, no histopathological findings were observed in the nose or lungs of mice following exposure to 4-nitrophenol for 78 weeks (3 days/week) at doses up to 160 mg/kg/day (NTP, 1993).

Intraperitoneal injection of nitrophenols in rats has shown a potential to alter respiration (Cameron 1958; Grant 1959). Rats were injected with 667 mg/kg of 2-nitrophenol, 250 mg/kg of 3-nitrophenol, 78 mg/kg of 4-nitrophenol, or saline control. Following injection, respiration rates were increased by 24% in the 3-nitrophenol group and 31% in the 2-nitrophenol group, compared to baseline; 4-nitrophenol respiration rate increase was comparable to saline control (2%) (Grant 1959). Oxygen consumption was decreased by 3-nitrophenol only, and 4-nitrophenol increased rate of carbon dioxide output (Cameron 1958).

2.5 CARDIOVASCULAR

No studies were identified regarding cardiovascular effects in humans after exposure to 2-, 3-, or 4-nitrophenol. In animals, no studies evaluated cardiovascular function; however, a limited number of

studies evaluated heart weight and/or histology following inhalation exposure to 2-nitrophenol and inhalation, oral, or dermal exposure to 4-nitrophenol. Based on available data, the heart does not appear to be a sensitive toxicity target of nitrophenols in animals.

No exposure-related changes in heart weight or histology were observed in rats after intermittent exposure to 2-nitrophenol concentrations up to 61.5 mg/m^3 (Hazleton 1984). In inhalation studies evaluating 4-nitrophenol, no exposure-related changes in heart weight or histology were observed in rats following intermittent exposure to concentrations up to 2,133 mg/m^3 (Smith et al. 1988) or 29.18 mg/m^3 for 4 weeks (Hazleton 1983).

Oral and dermal studies also did not observe exposure-related changes in heart weight or histology in rats exposed to 4-nitrophenol at doses up to 1,000 mg/kg/day via gavage for 28 days (Koizumi et al. 2001), 140 mg/kg/day via gavage for 13 weeks (Hazleton 1989), or 250 mg/kg/day via dermal application for 20–24 weeks (U.S. Army 1985). In mice, no exposure-related changes in heart weight or histology were observed following dermal exposure to 4-nitrophenol at doses up to 160 mg/kg/day for 78 weeks (NTP 1993).

2.6 GASTROINTESTINAL

No studies were identified regarding gastrointestinal effects in humans after exposure to 2-, 3-, or 4-nitrophenol. In animals, a limited number of studies evaluated the gastrointestinal system following inhalation exposure to 2-nitrophenol and inhalation, oral, or dermal exposure to 4-nitrophenol.

Based on limited data, the gastrointestinal system does not appear to be a sensitive toxicity target of nitrophenols following inhalation exposure. No histopathological changes to the gastrointestinal system were observed in rats after intermediate-duration inhalation exposure to 2-nitrophenol at concentrations up to 61.5 mg/m^3 (Hazleton 1984). Similarly, inhalation exposure to 4-nitrophenol as not associated with histopathological changes in the gastrointestinal system in rats following acute-duration exposure to concentrations up to 2,133 mg/m^3 (Smith et al. 1988) or intermediate-duration exposure concentrations up to 29.18 mg/m^3 (Hazleton 1983).

There is limited evidence suggesting possible gastrointestinal effects after acute oral exposure to 4-nitrophenol. Tang et al. (2016) qualitatively described damage to the small intestine of male Wistar rats following gavage exposure to 200 mg/kg/day for 1 or 3 days, including loss of mucosal goblet cells and

necrosis and desquamation of intestinal epithelial cells. This finding did not persist after a 3-day recovery period; no other tissues of the gastrointestinal tract were evaluated. However, in longer-duration gavage studies, no histopathological changes were noted in the gastrointestinal tract of Sprague-Dawley rats at doses up to 1,000 mg/kg/day for 28 days (Koizumi et al. 2001) or up to 140 mg/kg/day for 13 weeks (Hazleton 1989). The reason for this inconsistency with acute data is unclear; however, differences in strain susceptibility may have contributed to conflicting findings.

In the only dermal study evaluating the gastrointestinal system, no exposure-related lesions were observed in mice intermittently exposed to doses up to 160 mg/kg/day for 78 weeks (NTP 1993).

2.7 HEMATOLOGICAL

No studies were identified regarding hematological effects in humans after exposure to 2-, 3-, or 4-nitrophenol. In animals, a limited number of studies evaluated hematological indices following inhalation exposure to 2- or 4-nitrophenol and oral exposure to 4-nitrophenol. Based on a systematic evaluation of the literature, hematological effects are not classifiable as health effects following inhalation exposure to 4-nitrophenol. The full results of the systematic review are presented in Appendix C.

Acute inhalation studies indicate that exposure to 4-nitrophenol may be associated with methemoglobinemia. In a 2-week study with 4-nitrophenol, methemoglobin levels were increased in male rats exposed to 294 mg/m^3 (0.87%) and 2,133 mg/m^3 (1.53%), compared to the control value of 0.2% (Smith et al. 1988). The study authors also noted qualitatively that increased methemoglobinemia was accompanied by the darker urine and proteinuria. When Smith et al. (1988) repeated the experiment with lower concentrations, methemoglobin levels were increased at 112 mg/m^3 (1.5%) compared to control (0.5%); methemoglobin levels at 26 mg/m^3 (0.3%) were comparable to control. After a 14-day recovery period, methemoglobin levels remained elevated at 2,133 mg/m^3 only. In a 4-hour acute lethality study, methemoglobin levels were measured in two rats exposed to 1,304 mg/m^3 (Smith et al. 1988). At the end of the 14-day observation period, methemoglobin levels were reported as “normal;” however, limited conclusions can be drawn from this due to lack of control animals, small animal numbers, and measurement only at the end of the observation period.

There is no clear evidence for methemoglobinemia in rats following intermediate-duration exposure to nitrophenols. In a 4-week inhalation study in rats exposed to 2-nitrophenol, elevated methemoglobin levels (2.32% in males and 4.08% in females) were only observed at the lowest exposure level (5 mg/m^3)

at the interim blood draw on day 15; control values on day 15 were 1.00% in males and 1.99% in females (Hazleton 1984). At the end of the 4-week exposure, methemoglobin levels in rats were comparable to control at concentrations up to 61.5 mg/m^3. A similar non-monotonic trend was observed in male rats exposed to 4-nitrophenol for 4 weeks, which showed increased methemoglobin levels (2.23%) at the mid-exposure levels of 5.27 mg/m^3, compared to control (0.77%), but not at the high-exposure level of 29.18 mg/m^3 (1.11%); methemoglobinemia was not observed in female rats at concentrations up to 29.18 mg/m^3 (Hazleton, 1983). Additionally, Hazleton (1983) tested rats in three separate "squads," and methemoglobin levels varied widely between squads. Due to lack of an exposure-response relationship in these studies, findings are considered spurious and non-adverse.

No adverse effects in other hematological parameters were noted in rats following inhalation exposure to 4-nitrophenol at acute-duration concentrations up to 2,133 mg/m^3 (Smith et al. 1988) or intermediate-duration concentrations up to 29.18 mg/m^3 (Hazleton, 1983). Similarly, no adverse effects in other hematological parameters were observed in rats exposed to 2-nitrophenol at inhalation concentrations up to 61.5 mg/m^3 (Hazleton 1984).

Data pertaining to hematological effects following oral exposure to 4-nitrophenol are limited but do not report any adverse changes in hematological indices in rats following a single gavage exposure to 100 mg/kg (Abu-Qare et al. 2000), gavage exposure to 200 mg/kg/day for 14 days (Koizumi et al. 2001), or gavage exposure to doses up to 140 mg/kg/day for 13 weeks (Hazleton 1989).

2.8 MUSCULOSKELETAL

No studies were identified regarding musculoskeletal effects in humans after exposure to 2-, 3-, or 4-nitrophenol. In animals, a limited number of studies evaluated the musculoskeletal system following inhalation exposure to 2-nitrophenol and inhalation, oral, and dermal exposure to 4-nitrophenol. Based on available data, the musculoskeletal system does not appear to be a toxicity target of nitrophenols in animals.

No histopathological changes in skeletal muscle or bone were observed in rats following a 4-week exposure to 2-nitrophenol at concentrations up to 61.5 mg/m^3 (Hazleton 1984) or 4-nitrophenol at concentrations up to 29.18 mg/m^3 (Hazleton 1983). Similarly, no histopathological changes in skeletal muscle or bone were observed in rats exposed to 4-nitrophenol via gavage doses up to 140 mg/kg/day for 13 weeks (Hazleton 1989) or dermal doses up to 160 mg/kg/day for 78 weeks (NTP 1993).

2.9 HEPATIC

No studies were identified regarding hepatotoxicity in humans after exposure to 2-, 3-, or 4-nitrophenol. In animals, the hepatic system was evaluated following inhalation exposure to 2-nitrophenol and inhalation, oral, and dermal exposure to 4-nitrophenol.

In inhalation studies, no adverse, exposure-related changes in liver clinical chemistry, liver weight, or histology were observed in rats following a 4-week exposure to 2-nitrophenol at concentrations up to 61.5 mg/m^3 (Hazleton 1984) or 4-nitrophenol at concentrations up to 29.18 mg/m^3 (Hazleton 1983).

There is limited evidence suggesting possible hepatic effects after acute-duration oral exposure to 4-nitrophenol. Li et al. (2017) qualitatively reported disordered hepatocytes and detachment of the central vein of the hepatic lobule in male Wistar rats following a single gavage exposure to 200 mg/kg. Similarly, disordered hepatocytes and a widened hepatic were qualitatively reported in male rats exposed to 200 mg/kg/day via gavage for 3 days (Li et al. 2017). Absolute and relative liver weights were decreased by 34 and 12%, respectively, after the 3-day exposure to 200 mg/kg/day (Li et al. 2017; Tang et al. 2016). Organ weight decreases and histopathological changes were transient, showing reversal following a 3-day recovery period. Li et al. (2017) proposed that hepatic effects were mediated via the aryl hydrocarbon receptor (AhR) signaling pathway. In support, a 36% increase in AhR levels were observed in the liver of Sprague-Dawley rats exposed to 100 mg/kg/day via subcutaneous injection for 28 days (Chen et al. 2016). The injection study also indicated that 4-nitrophenol exposure induces oxidative stress in the liver, based on elevated levels of superoxide dismutase and catalase. Other effects noted following exposure to 100 mg/kg/day via subcutaneous injection include histopathological changes (inflammatory cell infiltration, hepatocyte degeneration, and hepatic sinusoid damage) and mild elevations of alanine aminotransferase (ALT; 28%), aspartate aminotransferase (AST; 56%), and total bilirubin (56%). No changes in liver weight were observed.

In contrast to acute oral studies, no adverse effects on liver clinical chemistry, weight, or histology were observed in Sprague-Dawley rats exposed to 4-nitrophenol at gavage doses up to 1,000 mg/kg/day for 28 days (Koizumi et al. 2001) or up to 140 mg/kg/day for 13 weeks (Hazleton 1989). The reason for this inconsistency with acute data is unclear; however, differences in strain susceptibility may have contributed to conflicting findings,

In dermal studies, 4-nitrophenol exposure was not associated with adverse changes in liver weight or histology in rats at doses up to 250 mg/kg/day for 20–24 weeks (U.S. Army 1985) or in mice at doses up to 160 mg/kg/day for 78 weeks (NTP 1993).

2.10 RENAL

No studies were identified regarding renal effects in humans after exposure to 2-, 3-, or 4-nitrophenol. In animals, the renal system was evaluated following inhalation, oral, and dermal exposure to 4-nitrophenol and inhalation exposure to 2-nitrophenol. Based on available data, the renal system does not appear to be a sensitive toxicity target of nitrophenols in animals following inhalation or dermal exposure; findings following oral exposure are mixed.

No exposure-related changes in kidney serum biochemistry, weight, or histology were observed in rats intermittently exposed to 4-nitrophenol concentrations up to 2,133 mg/m^3 for 2 weeks (Smith et al. 1988), 4-nitrophenol concentrations up to 29.18 mg/m^3 for 4 weeks (Hazleton 1983), or 2-nitrophenol concentrations up to 61.5 mg/m^3 for 4 weeks (Hazleton 1984). A 38% decrease in urine volume was reported in rats exposed to 2,133 mg/m^3 for 2 weeks (Smith et al. 1988). The biological relevance of this finding in the absence of biochemical or histopathological changes in unclear. It could be secondary to decreased water intake since rats were lethargic at this exposure concentration; however, water consumption was not measured.

One acute oral study reported a 14% increase in relative kidney gland weight in rats exposed to 200 mg/kg/day via gavage for 3 days; however, this finding was considered secondary to a 25% decrease in body weight and therefore not adverse (Li et al. 2017; Tang et al. 2016). In support, both body weight and relative kidney weights were comparable to control after a 3-day recovery period. The same study authors did not observe an exposure-related change in kidney gland weight following a single exposure to 200 mg/kg. This study did not evaluate renal clinical chemistry or kidney histology following exposure, and no other acute-duration oral studies evaluated renal endpoints.

One intermediate-duration oral study reported eosinophilic bodies in the proximal renal tubular cells in Sprague-Dawley male rats, but not female rats, exposed to 4-nitrophenol at gavage doses ≥400 mg/kg/day for 28 days (Koizumi et al. 2001). The study authors concluded that the male-rat specific change in the kidney is due to α2u-globulin formation, which is not relevant to humans. However, human relevance cannot be ruled out based on the available analysis, which did not evaluate kidneys for α2u-globulin

accumulation or report hyaline droplet formation or other aspects of the established pathological sequence associated with α2u-globulin nephropathy (e.g., single cell necrosis, sloughing of epithelial cells into the proximal tubule, formation of granular casts, linear mineralization of the papilla, or tubule hyperplasia or regeneration; as established by EPA [1991]). Koizumi et al. (2001) did not observe any exposure-related changes in renal clinical chemistry or kidney weight following exposure to doses up to 1,000 mg/kg/day. In another intermediate-duration oral study evaluating 4-nitrophenol, no exposure-related changes in kidney serum biochemistry, weight, or histology were observed in male or female Sprague-Dawley rats at gavage doses up to 140 mg/kg/day for 13 weeks (Hazleton 1989).

In a 2-generation dermal studies, no exposure-related changed in kidney weight or histology were observed in F0 or F1 rats exposed to 4-nitrophenol at doses up to 250 mg/kg/day for 20–24 weeks per generation (U.S. Army 1985). Similarly, no exposure-related renal lesions were observed mice exposed to 4-nitrophenol at dermal doses up to 160 mg/kg/day for 78 weeks (NTP 1993).

2.11 DERMAL

No studies were identified regarding dermal effects in humans after exposure to 2-, 3-, or 4-nitrophenol. In animals, dermal effects were evaluated following inhalation exposure to 2-nitrophenol and inhalation, oral, and dermal exposure to 4-nitrophenol.

The skin is not a target of nitrophenol toxicity following inhalation or oral exposure. No dermatological or histopathological skin changes were observed in rats after intermittent exposure to 2-nitrophenol concentrations up to 61.5 mg/m^3 for 4 weeks (Hazleton 1984) or intermittent exposure to 4-nitrophenol concentrations up to 2,133 mg/m^3 for 2 weeks (Smith et al. 1988) or 29.18 mg/m^3 for 4 weeks (Hazleton 1983). Similarly, no dermatological or histopathological skin changes were observed in rats exposed to 4-nitrophenol via gavage for 13 weeks (Hazleton 1989).

Acute dermal exposure to 4-nitrophenol in rabbits is associated with skin irritation. Erythema and edema were reported in rabbits exposed to 148 mg/kg for 4 hours or 5,000 mg/kg for 24 hours (Branch et al. 1983a; Monsanto 1984); additional effects noted at 5,000 mg/kg included a dark brown discoloration of the dermal tissue, sloughing and scarring of the skin, hardening of the skin of the exposed area, and epidermal desquamation. Another study reported skin scabbing and scarring in rabbits exposed to 184 mg/kg for 24 hours (Monsanto 1983b).

In rats, repeated dermal application of ≥50 mg/kg/day in a 2-generation study (20–24 weeks, 5 days/week) resulted in skin irritation (erythema, scaling, scabbing, cracking) and histopathological changes in the skin (chronic inflammation, acanthosis, eschar, sebaceous hypertrophy) in F0 and F1 rats (U.S. Army 1985). However, no dermal effects were observed in mice following dermal application of 4-nitrophenol for 78 weeks (3 days/week) at doses up to 160 mg/kg/day (NTP 1993).

2.12 OCULAR

No studies were identified regarding ocular effects in humans after exposure to 2-, 3-, or 4-nitrophenol. In animals, ocular effects were evaluated following inhalation, oral, and direct ocular exposure to 4-nitrophenol and inhalation exposure to 2-nitrophenol. Based on a systematic evaluation of the literature, ocular effects are a suspected health effect of exposure to 4-nitrophenol. The full results of the systematic review are presented in Appendix C.

Inhalation exposure to 4-nitrophenol has been associated with ocular effects in rats. Transient corneal opacity was observed in 4/6 rats following a single 4-hour exposure to 4,059 mg/m^3; the effect persisted in one rat throughout a 14-day observation period (Smith et al. 1988). No clinical evidence of ocular effects or histopathological changes in the eye were observed after a 4-hour exposure to 1,304 mg/m^3 or repeated exposure (6 hours/day, 5 days/week) for 2 weeks at concentrations up to 2,133 mg/m^3 (Smith et al. 1988). Intermittent exposure for 4 weeks resulted in an increased incidence of unilateral and bilateral diffused anterior capsular cataracts in male rats at 29.18 mg/m^3 (6/15), compared to 1/15 control male rats (Hazleton 1983). While 6/15 female rats at 29.18 mg/m^3 also had cataracts, incidence was not significantly increased over control female incidence (4/15). Cataracts are likely to have been caused by direct ocular contact with 4-nitrophenol dust; however, a systemic effect cannot be totally excluded in the absence of mechanistic data.

No exposure-related changes ophthalmology or eye histology were observed in rats intermittently exposed to 2-nitrophenol via inhalation at concentrations up to 61.5 mg/m^3 (Hazleton 1984) or in rats exposed to 4-nitrophenol at gavage doses up to 140 mg/kg/day for 13 weeks (Hazleton 1989). No histopathological changes in the eye were observed in mice following 4-nitrophenol exposure to dermal doses up to 160 mg/kg/day for 78 weeks (NTP 1993).

Severe conjunctival irritation and blistering, moderate-to-severe corneal cloudiness, corneal neovascularization, and inflammation and visible destruction of the iris were observed in rabbits

following ocular instillation of 4-nitrophenol (as a granular solid) directly into the conjunctival sac at doses of 70–100 mg/kg (EPA 1992b; Monsanto 1983a). Findings were not reversible in most animals during the post-observation period of 7 days (EPA 1992b) or 31 days (Monsanto 1983a). EPA (1992b) classified 4-nitrophenol as a corrosive substance based on these results.

2.13 ENDOCRINE

No studies were identified regarding endocrine system effects in humans after exposure to 2-, 3-, or 4-nitrophenol. In animals, a limited number of studies evaluated the endocrine system following inhalation exposure to 2-nitrophenol and inhalation, oral, and dermal exposure to 4-nitrophenol. Based on available data, the endocrine system does not appear to be a sensitive toxicity target of nitrophenols in animals.

No histopathological changes were noted in the thyroid gland in rats intermittently exposed to 4-nitrophenol at inhalation concentrations up to 2,133 mg/m^3 for 2 weeks (Smith et al. 1988). In 4-week inhalation studies in rats, no exposure-related changes in organ weight or histology of the adrenal glands, thyroid, pituitary gland, or pancreas were observed following intermittent exposure to 2-nitrophenol at concentrations up to 61.5 mg/m^3 (Hazleton 1984) or 4-nitrophenol at concentrations up to 29.18 mg/m^3 (Hazleton 1983).

One acute oral study reported a 41% increase in relative adrenal gland weight in rats exposed to 200 mg/kg/day via gavage for 3 days; however, this finding was considered secondary to a 25% decrease in body weight and therefore not adverse (Li et al. 2017; Tang et al. 2016). In support, both body weight and relative adrenal weights were comparable to control after a 3-day recovery period. The same study authors did not observe an exposure-related change in adrenal gland weight following a single exposure to 200 mg/kg. In intermediate-duration oral studies, no exposure-related changes in organ weight or histology of the adrenal glands, thyroid, pituitary gland, or pancreas were observed in rats following gavage exposure to 4-nitrophenol at doses up to 1,000 mg/kg/day for 28 days (Koizumi et al. 2001) or 140 mg/kg/day for 13 weeks (Hazleton 1989).

In a chronic-duration dermal study, no exposure-related histopathological lesions were observed in the adrenal glands, thyroid, parathyroid, pituitary gland, or pancreas of mice following dermal application of doses up to 160 mg/kg/day for 78 weeks (NTP 1993).

2.14 IMMUNOLOGICAL

No studies were identified regarding immunotoxicity in humans after exposure to 2-, 3-, or 4-nitrophenol. In animals, a limited number of studies evaluated immune system organs following inhalation, oral, and dermal exposure to 4-nitrophenol and inhalation exposure to 2-nitrophenol. Based on available data, the immune system does not appear to be a sensitive toxicity target of nitrophenols in animals; however, none of the identified animal studies evaluated functional immune endpoints.

Absolute spleen weight was reportedly decreased in rats intermittently exposed to 4-nitrophenoal at an inhalation concentration of 2,133 mg/m^3 for 2 weeks; however, the toxicological relevance is uncertain due to unspecified magnitude of change and no associated histopathological changes (Smith et al. 1988). For other immune organs, no exposure-related changes were noted in thymus weight or histology or bone marrow histology at concentrations up to 2,133 mg/m^3 for 2 weeks (Smith et al. 1988). In 4-week inhalation studies in rats, no exposure-related changes were observed for spleen or thymus weight or spleen, thymus, bone marrow, or lymph node histology following intermittent exposure to 2-nitrophenol at concentrations up to 61.5 mg/m^3 (Hazleton 1984) or 4-nitrophenol at concentrations up to 29.18 mg/m^3 (Hazleton 1983).

In an acute-duration oral study, no exposure-related changes in spleen weight were observed following exposure to 4-nitrophenol at 200 mg/kg/day via gavage for 1 or 3 days; histology was not evaluated (Tang et al. 2016). In intermediate-duration oral studies, no exposure-related changes in spleen or thymus weight or spleen, thymus, bone marrow, or lymph node histology were observed in rats following gavage exposure to 4-nitrophenol at doses up to 1,000 mg/kg/day for 28 days (Koizumi et al. 2001) or 140 mg/kg/day for 13 weeks (Hazleton 1989).

In a chronic-duration dermal study, no exposure-related histopathological lesions were observed in the spleen, thymus, bone marrow, or lymph nodes of mice following dermal application of doses up to 160 mg/kg/day for 78 weeks (NTP 1993).

2.15 NEUROLOGICAL

No studies were identified regarding neurotoxicity in humans after exposure to 2-, 3-, or 4-nitrophenol. In animals, neurological effects were evaluated following inhalation, oral, and dermal exposure to 4-nitrophenol and inhalation exposure to 2-nitrophenol.

In an acute-duration inhalation study, lethargy was "sporadically" observed in rats during the second week of exposure to 4-nitrophenol at a concentration of 2,133 mg/m^3 4-nitrophenol (5 days/week); no clinical signs of neurotoxicity were observed at concentrations ≤294 mg/m^3 (Smith et al. 1988). No histopathological changes in the brain were observed. In 4-week inhalation studies in rats, no exposure-related changes in brain weight or histology were observed following intermittent exposure to 2-nitrophenol at concentrations up to 61.5 mg/m^3 (Hazleton 1984) or 4-nitrophenol at concentrations up to 29.18 mg/m^3 (Hazleton 1983).

As discussed in Section 2.2 (Death), clinical signs of neurotoxicity were often reported prior to death following exposure to lethal oral doses of 4-nitrophenol. Convulsions and prostration were observed following single gavage exposures to ≥268 mg/kg (Branch et al. 1983b). In intermediate-duration gavage studies, neurological effects noted prior to death included convulsions in neonatal rats exposed to ≥230 mg/kg/day on PNDs 4–21 (Koizumi et al. 2001); decreased locomotor activity, convulsions, and prostration in rats exposed to 1,000 mg/kg/day for up to 28 days (Koizumi et al. 2001); and prostration prior to death in rats exposed to 140 mg/kg/day for up to 13 weeks (Hazleton 1989). No clinical signs of neurotoxicity were noted at non-lethal doses in acute- or intermediate-duration oral studies.

No exposure-related changes in brain weight were noted in rats following a single gavage exposure to 100 mg/kg (Abu-Qare et al. 2000) and no exposure-related changes in brain weight or histology were observed in rats exposed to gavage doses up to 1,000 mg/kg/day for 28 days (Koizumi et al. 2001) or 140 mg/kg/day for 13 weeks (Hazleton 1989)

In dermal studies evaluating 4-nitrophenol, no exposure-related clinical signs of neurotoxicity were observed in adult F0 or F1 rats exposed to concentrations up to 250 mg/kg/day over two generations (20–24 weeks per generation) or in mice exposed to concentrations up to 160 mg/kg/day for 78 weeks (NTP 1993; U.S. Army 1985). A 15% decrease in relative brain weight was reported in adult F1 males at 250 mg/kg/day in the 2-generation study; however, this is considered secondary to a concurrent 11% increase in F1 male body weight (U.S. Army 1985). Brain weights in adult F0 males and females and adult F1 females were comparable to control at doses up to 250 mg/kg/day. No exposure-related changes in brain weight or histology were observed in the chronic-duration study in mice at doses up to 160 mg/kg/day (NTP 1993).

2.16 REPRODUCTIVE

No studies were identified regarding reproductive toxicity in humans after exposure to 2-, 3-, or 4-nitrophenol. In animals, the organs of the reproductive system were evaluated following inhalation and oral exposure to 2-nitrophenol and inhalation, oral, and dermal exposure to 4-nitrophenol. Female reproductive function was evaluated in oral and dermal studies, while male reproductive function was only evaluated in a dermal study. Available studies do not indicate that the reproductive system is a sensitive target of nitrophenol toxicity.

The only study identified evaluating both male and female reproductive function following exposure to 4-nitrophenol was a 2-generation dermal study in rats, which found no evidence of impaired reproductive function at doses up to 250 mg/kg/day (U.S. Army 1985). No exposure-related changes were observed in mating, fertility, gestation, or litter indices. Additionally, no exposure-related changes were observed in reproductive organ weight or histology.

Acute oral studies evaluated potential reproductive effects in rodent dams exposed to 2- or 4-nitrophenol during gestation. A 1.9-fold increase in post implantation loss was observed in rat dams exposed to 1,000 mg/kg/day of 2-nitrophenol via gavage from GDs 6–15 (Laughlin et al. 1983). Observed mean post implantation loss was 2.3±2.2 at 1,000 mg/kg/day, compared to 1.2±0.8 in concurrent controls. While this finding is not statistically significant due to high standard deviation and small animal number, the study authors concluded that observed post implantation losses may be biologically relevant since values were outside the upper range for historical controls. No changes in post implantation losses were observed at doses up to 500 mg/kg/day. In acute-duration gestational exposure studies evaluating 4-nitrophenol, no exposure-related changes in corpora lutea, implantations, implantation loss or resorption, or number of viable fetuses was observed at doses up to 100 mg/kg/day in rats (Abu-Qare et al. 2000; EPA 1992a) or 400 mg/kg/day in mice (Plasterer et al. 1985).

Other studies that evaluated weight and/or histology of male and female reproductive organs, but not reproductive function, did not identify the reproductive system as a toxicity target of nitrophenols. No exposure-related changes in testes weight or testes or epididymides histology were observed in in rats intermittently exposed to 4-nitrophenol at inhalation concentrations up to 2,133 mg/m^3 for 2 weeks (Smith et al. 1988). In 4-week inhalation studies in rats, no exposure-related changes in the weight or histology of male or female reproductive organs were observed following intermittent exposure to 2-nitrophenol at concentrations up to 61.5 mg/m^3 (Hazleton 1984) or 4-nitrophenol at concentrations up to 29.18 mg/m^3

(Hazleton 1983). Similarly, no exposure-related changes in male or female reproductive organ weight or histology were observed in rats exposed to 4-nitrophenol at gavage doses up to 1,000 mg/kg/day for 28 days (Koizumi et al. 2001) or 140 mg/kg/day for 13 weeks (Hazleton 1989). One acute oral study also reported a lack of exposure-related changes in testes weight in rats exposed to a 4-nitrophenol dose of 200 mg/kg/day for 1 or 3 days (Tang et al. 2016). Mice exposed to 4-nitrophenol at dermal doses up to 160 mg/kg/day for 78 weeks also showed no evidence of histopathological changes in male or female reproductive organs (NTP 1993).

Although inhalation, oral, and dermal exposure data do not indicate that the reproductive system is a sensitive target of nitrophenol toxicity, 4-nitrophenol has been shown to alter reproductive endpoints after parenteral exposure in male rodents. Reported effects include increased serum testosterone, decreased serum estradiol, and hyperplasia of Leydig cells in the testes of rats following daily subcutaneous injections of 10 mg/kg/day for 4 weeks (Zhang et al. 2013); decreased serum testosterone and sperm count in rats following daily subcutaneous injections of 100 mg/kg/day for 4 weeks (Zhang et al. 2015); and severe damage to the seminiferous tubules in mice exposed to 4-nitrophenol via weekly intraperitoneal injections of 50 mg/kg/day for 6 weeks (Mi et al. 2013). These changes were associated with altered expression of estrogen receptors (ERs; increased ERα and decreased ERβ) in rats (Zhang et al. 2013) and elevated markers of oxidative stress in rats and mice (Mi et al. 2013; Zhang et al. 2015). Markers of oxidative stress were also elevated in rat testes following a single intratesticular injection of 3 mg/kg of 4-nitrophenol (Zhang et al. 2016). In cultured testicular somatic cells from mice, no evidence of oxidative stress was observed following exposure to 4-nitrophenol; however, decreased cell proliferation, increased apoptosis, and altered cell cycles were observed (Wei et al. 2021). Given the lack of corroborating evidence of these observed reproductive outcomes in studies using the oral, inhalation, and dermal routes of exposure, the human relevance of male reproductive toxicity following parenteral exposure are unclear.

2.17 DEVELOPMENTAL

No studies were identified regarding developmental toxicity in humans after exposure to 2-, 3-, or 4-nitrophenol. In animals, a limited number of studies evaluated developmental effects following oral and dermal exposure to 4-nitrophenol.

The available literature on acute oral exposure to 4-nitrophenol has not identified any associations with developmental effects following gestational exposure. The only comprehensive teratology study

identified did not observe exposure-related changes in litter parameters, fetal body weight, or fetal variations or malformations were observed in rats following maternal exposure to doses up to 27.6 mg/kg/day from GDs 6–16 (EPA 1992a). Other developmental studies did not observe exposure-related effects on offspring weight or gross examination in rats following single maternal exposures up to 1,000 mg/kg (Abu-Qare et al. 2000; Kavlock 1990) or in mice following maternal exposure to doses up to 400 mg/kg/day on GDs 7–14 (Plasterer et al. 1985). While Kavlock (1990) classified 4-nitrophenol as an active developmental toxicant based on a >10% reduction in litter biomass following maternal exposure to gavage doses ≥667 mg/kg on GD 11, the reported statistical analysis in this study do not support this conclusion.

One oral study evaluated growth and development in rats following neonatal exposure to 4-nitrophenol at gavage doses up to 160 mg/kg/day on PNDs 4–21 (Koizumi et al. 2001). Some rats were sacrificed immediately after exposure, while others were maintained (without further exposure) until 12 weeks of age to evaluate sexual development. No exposure-related changes in general behavior, body weight, reflex ontogeny, developmental landmarks, hematology, clinical chemistry, gross necropsy, organ weight or histology, or timing of puberty (preputial separation or vaginal opening) were observed. In a dose-range-finding study using the same dosing schedule, increased mortality and convulsions were observed during the neonatal exposure period at ≥230 mg/kg/day (Koizumi et al. 2001).

No developmental effects in F1 or F2 offspring were observed in a 2-generation dermal study in rats at doses of 4-nitrophenol up to 250 mg/kg/day for 20–24 weeks per generation (U.S. Army 1985). Survivability from birth to weaning was effectively 100% for rat pups in all dosage groups in both the F1 and F2 generations, and all F2 pups that had not been directly dosed with 4-nitrophenol were normal in appearance, behavior, and growth (U.S. Army 1985).

Although oral and dermal exposure data do not indicate that the developing rodent is a sensitive target of nitrophenol toxicity, subcutaneous exposure to 4-nitrophenol has been shown to alter reproductive development in female rats and exhibit anti-androgenic and estrogenic activities in male and female rats following exposure prior to puberty. A single subcutaneous injection of 4-nitrophenol at 10 mg/kg in female neonatal rats on PND 0 resulted in an increase in the ratio of primordial and primary follicles in female rats at PNDs 14 and 21, an increase in serum estradiol and luteinizing hormone (LH) at PND 14 (but not PND 7 or PND 21), a decrease in ERβ expression in the ovaries on PND 7 and PND 14, and a 2–3-day delay in vaginal opening (Zhang et al. 2017). In prepubertal ovariectomized rats, exposure to ≥10 mg/kg/day via subcutaneous injections on PNDs 25–31 increased uterine weights; no changes in

serum follicle-stimulating hormone (FSH) or LH were observed at doses up to 100 mg/kg/day (Li et al. 2006). In prepubertal, testosterone-supplemented, castrated male rats, exposure to 0.1 mg/kg/day via subcutaneous injections on PNDs 28–31 resulted in an increase in serum LH and FSH levels and a decrease in weights of androgen-dependent accessory sex glands (seminal vesicles, ventral prostate, glans penis, levator ani plus bulbocavernosus muscles). Given the lack of corroborating evidence for altered reproductive development in oral and dermal studies, the human relevance of developmental reproductive toxicity following subcutaneous injection are unclear.

2.18 CANCER

No studies were identified regarding cancer in humans after exposure to 2-, 3-, or 4-nitrophenol. Animal studies evaluating potential carcinogenicity of nitrophenols are limited to dermal studies evaluating rodents exposed to 2- or 4-nitrophenol.

There is only one study of complete carcinogenicity of 4-nitrophenol (NTP 1993). In this study, a comprehensive histopathological examination did not show any exposure-related increases in malignant or benign neoplasms in mice at dermal doses up to 160 mg/kg/day, 3 times/week for 78 weeks. When benign and malignant neoplasms was combined, the overall incidence was elevated in male mice exposed to 40 mg/kg/day (33/59) and 80 mg/kg/day (33/60), compared to control (20/56); however, the overall incidence of benign and malignant neoplasms combined at 160 mg/kg/day was comparable to control (20/57). NTP (1993) concluded that there was no evidence of carcinogenic activity in male or female Swiss-Webster mice exposed dermally to 4-nitrophenol doses up to 160 mg/kg/day.

A 2-generation study in rats indicated that exposure to 4-nitrophenol at doses up to 250 mg/kg/day for 20–24 weeks per generation did not result in an increase in grossly identified neoplasms; however, a comprehensive histopathological examination was not conducted (U.S. Army 1985).

In a series of initiation-promotion dermal studies, a 20% solution of 2- or 4-nitrophenol dissolved in dioxane was applied to the shaved skin of mice twice weekly for 12 weeks to evaluate potential tumor-promoting activity (Boutwell and Bosch 1959). No skin tumors were observed; however, the utility of this study is limited due to lack of a dioxane-only control and lack of evaluation following exposure to an initiating compound.

IRIS (2002), IARC (2022), and NTP (2021) have not evaluated the potential for 2-, 3-, or 4-nitrophenol to cause carcinogenicity in humans.

2.19 GENOTOXICITY

Available data generally indicate that 2-, 3-, and 4-nitrophenol are not mutagenic. Findings for clastogenicity and deoxyribonucleic acid (DNA) damage are mixed but suggest that nitrophenols may be clastogenic or interact directly with DNA under certain conditions.

2-Nitrophenol is not mutagenic in *Salmonella typhimurium* in the presence or absence of metabolic activation (Chiu et al. 1978; Dellarco and Prival 1989; Shimizu and Yano 1986; Suzuki et al. 1983) or in *Escherichia coli* in the absence of metabolic activation (Szybalski 1958). 2-Nitrophenol did not induce DNA damage in *Bacillus subtilis* (Shimizu and Yano 1986). However, in the *umu* test in *S. typhimurium,* one study reported that 2-nitrophenol was DNA damaging (Degirmenci et al. 2000) but a second study did not find evidence of DNA damage (Bonnefoy et al. 2012). No data were available regarding genotoxic properties of 2-nitrophenol in eukaryotic organisms. Results of *in vitro* genetic testing for 2-nitrophenol are presented in Table 2-4.

Table 2-4. Genotoxicity of 2-Nitrophenol *In Vitro*

Species (test system)	Endpoint	Results activation		Reference
		With	Without	
Prokaryotic organisms				
Salmonella typhimurium strains TA98, TA100	Gene mutation	NT	–	Chiu et al. 1978
S. typhimurium strains TA98, TA100	Gene mutation	–	–	Dellarco and Prival 1989
S. typhimurium strains TA98, TA1538, TA1537, TA100, TA1535	Gene mutation	–	–	Shimizu and Yano 1986
S. typhimurium strains TA98, TA100	Gene mutation	–	–	Suzuki et al. 1983
Escherichia coli strain sd-4-73	Gene mutation	NT	–	Szybalski 1958
S. typhimurium strain TA1535/pSK1002	DNA damage	–	–	Bonnefoy et al. 2012

Table 2-4. Genotoxicity of 2-Nitrophenol *In Vitro*

Species (test system)	Endpoint	Results activation		Reference
		With	Without	
S. typhimurium strain TA1535/pSK1002	DNA damage		+[a]	Degirmenci et al. 2000
Bacillus subtilis strains H17, M45	DNA damage	NT	–	Shimizu and Yano 1986

[a]Test was conducted with and without metabolic activation; however, the study authors did not indicate under which condition(s) 2-nitrophenol was positive for DNA damage.

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

3-Nitrophenol is not mutagenic in *S. typhimurium* in the presence or absence of metabolic activation (Haworth et al. 1983; Shimizu and Yano 1986; Suzuki et al. 1983) or in *E. coli* in the absence of metabolic activation (Szybalski 1958). 3-Nitrophenol induced DNA damage in *B. subtilis* (Shimizu and Yano 1986) and *S. typhimurium* (Degirmenci et al. 2000). No data were available regarding genotoxic properties of 3-nitrophenol in eukaryotic organisms. Results of *in vitro* genetic testing for 3-nitrophenol are presented in Table 2-5.

Table 2-5. Genotoxicity of 3-Nitrophenol *In Vitro*

Species (test system)	Endpoint	Results activation		Reference
		With	Without	
Prokaryotic organisms				
Salmonella typhimurium strains TA1535, 1537, TA98, TA100	Gene mutation	–	–	Haworth et al. 1983
S. typhimurium strains TA98, TA1538, TA1537, TA100, TA1535	Gene mutation	–	–	Shimizu and Yano 1986
S. typhimurium strains TA98, TA100	Gene mutation	–	–	Suzuki et al. 1983
Escherichia coli strain sd-4-73	Gene mutation	NT	–	Szybalski 1958
Bacillus subtilis strains H17, M45	DNA damage	NT	+	Shimizu and Yano 1986
S. typhimurium strain TA1535/pSK1002	DNA damage		+[a]	Degirmenci et al. 2000

[a]Test was conducted with and without metabolic activation; however, the study authors did not indicate under which condition(s) 3-nitrophenol was positive for DNA damage.

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

Results of *in vitro* and in vivo genetic testing for 4-nitrophenol are presented in Tables 2-6 and 2-7, respectively.

Table 2-6. Genotoxicity of 4-Nitrophenol *In Vitro*

Species (test system)	Endpoint	Results activation		Reference
		With	Without	
Prokaryotic organisms				
Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, TA100	Gene mutation	–	(+) TA1535 – TA1537, TA1538, TA98, TA100	EPA 1990b
Salmonella typhimurium strains TA98, TA100	Gene mutation	–	–	Dellarco and Prival 1989
S. typhimurium strains TA1535, 1537, TA98, TA100	Gene mutation	–	–	Haworth et al. 1983
S. typhimurium strains TA100, TA1535, TA1537, TA98	Gene mutation	–	–	NTP 1993
S. typhimurium strains G46, TA1535, TA1000, C3076, TA1537, D3052, TA1538, TA98	Gene mutation	–	–	Probst et al. 1981
S. typhimurium strains TA98, TA1538, TA1537, TA100, TA1535	Gene mutation	–	–	Shimizu and Yano 1986
Salmonella typhimurium strains TA98, TA100)	Gene mutation	–	–	Suzuki et al. 1983
Escherichia coli strains WP2, WP2 uvrA	Gene mutation	–	–	Probst et al. 1981
Escherichia coli strain sd-4-73	Gene mutation	–	–	Szybalski 1958
E. coli strain K12 envA uvrB	Prophage induction	–	NT	Ho and Ho 1981
S. typhimurium strains TA1538, TA1978	DNA repair	NT	–	Rashid and Mumma 1986
E. coli K12 and WP2 strains	DNA repair	NT	–	Rashid and Mumma 1986
Bacillus subtilis strains H17, M45	DNA damage	NT	+	Shimizu and Yano 1986
Proteus mirabilis strains PG273, PG713	DNA damage	NT	(+)	Adler et al. 1976
Mammalian organisms				
Mouse L5178Y lymphoma TK +/– cells	Gene mutation	–	NT	Amacher and Turner 1982
Mouse L5178Y lymphoma TK +/– cells	Gene mutation	–	–	Oberly et al. 1984
Mouse L5178Y lymphoma TK +/– cells	Gene mutation	–	–	EPA 1990c

Table 2-6. Genotoxicity of 4-Nitrophenol *In Vitro*

Species (test system)	Endpoint	Results activation		Reference
		With	Without	
Chinese hamster ovary cells	Chromosomal aberrations	–	+	EPA 1990a
Chinese hamster ovary cells	Chromosomal aberrations	+	–	NTP 1993
Chinese hamster ovary cells	Sister chromatid exchanges	–	–	NTP 1993
Rat primary hepatocytes	Unscheduled DNA synthesis	NT	–	Probst et al. 1981
Chinese hamster ovary cells	Inhibition of DNA synthesis	NT	+	Garrett and Lewtas 1983
Chinese hamster ovary cells	DNA damage	–	–	Hartmann and Speit 1997

+ = positive result; – = negative result; (+) = weakly positive result; DNA = deoxyribonucleic acid; NT = not tested

Table 2-7. Genotoxicity of 4-Nitrophenol *In Vivo*

Species (test system)	Endpoint	Results	Reference
Mouse (s.c.)	Host-mediated mutations	–	Buselmaier et al. 1973
Drosophila melanogaster (inhalation)	Sex-linked recessive lethal mutations	–	NTP 1993
D. melanogaster (oral)	Sex-linked recessive lethal mutations	–	NTP 1993

– = negative results; s.c. = subcutaneous injection

In almost all *in vitro* studies identified, 4-nitrophenol was not mutagenic in the presence or absence of metabolic activation in *S. typhimurium* (Dellarco and Prival 1989; Haworth et al. 1983; NTP 1993; Probst et al. 1981; Shimizu and Yano 1986; Suzuki et al. 1983), in the absence of metabolic activation in *E. coli* (Szybalski 1958), or in the presence or absence of metabolic activation in mouse lymphoma cells (Amacher and Turner 1982; EPA 1990c; Oberly et al. 1984). One study reported weak mutagenic activity (2.7-fold increase in revertants) in *S. typhimurium* strain TA1535 in the presence of metabolic activation only; all other strains were negative (EPA 1990b). 4-Nitrophenol did not induce prophage in *E. coli* (Ho and Ho 1981). *In vivo*, 4-nitrophenol did not induce host-mediated mutations in mice following subcutaneous injection (Buselmaier et al. 1973) or sex-linked recessive mutations in *Drosophila melanogaster* following inhalation or dietary exposure (NTP 1993).

Findings regarding clastogenicity following *in vitro* exposure to 4-nitrophenol are mixed. Two studies reported increased chromosomal aberrations in Chinese hamster ovary (CHO) cells following exposure to 4-nitrophenol; however, EPA (1990a) reported induction without metabolic activation (but not with metabolic activation) and NTP (1993) reported induction with metabolic activation (but not without metabolic activation). Both studies utilized rat S9 as the metabolic activation system. No induction of sister chromatid exchanges was observed in CHO cells exposed to 4-nitrophenol (NTP 1993).

Findings regarding DNA damage and repair in bacteria and mammalian cells following *in vitro* exposure to 4-nitrophenol are also mixed. Shimizu and Yano (1986) reported induced DNA damage when tested in *B. subtilis* by the rec assay, and Adler et al. (1976) reported a weak (1.35-fold) increase in DNA damage in repair-deficient *Proteus mirabilis* strain, compared to wild-type strain. However, DNA repair was not induced in *S. typhimurium* or *E. coli* (Rashid and Mumma 1986). In mammalian cells, 4-nitrophenol did not induce unscheduled DNA synthesis in rat primary hepatocytes (Probst et al. 1981) or DNA damage in CHO cells (Hartmann and Speit 1997). However, Garrett and Lewtas (1983) reported a 97% inhibition of DNA synthesis in CHO cells exposed to 4-nitrophenol in the absence of cytotoxicity.