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 nitrobenzene. 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 nitrobenzene, but may not be inclusive of the entire body of literature. 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; animal dermal studies are presented in Table 2-3.

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

Agency has established guidelines and policies that are used to classify these endpoints. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of nitrobenzene are indicated in Table 2-2 and Figure 2-3.

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

As illustrated in Figure 2-1, most of the health effects data for nitrobenzene comes from experimental animal studies. Even though there were more than 30 human studies, the majority of these were case studies. There were studies of comprehensive noncancer endpoints in animals exposed by inhalation and oral routes, and cancer was assessed in animals exposed by inhalation. The effects examined in most studies included reproductive, body weight, hematology, hepatic, renal, and neurological endpoints.

The human and animal studies suggest that the hematological, respiratory, hepatic, renal, endocrine, and reproductive systems are the most sensitive targets of nitrobenzene’s toxicity.

- ***Hematological:*** Nitrobenzene has induced methemoglobinemia in humans and animals through all exposure routes. In addition, animal studies of all exposure routes and durations have shown related effects including hemolytic anemia, large increases in spleen weight, and histopathology changes in the spleen (congestion, extramedullary hematopoiesis, hemosiderosis, lymphoid depletion) and bone marrow (hypercellularity).

- ***Respiratory:*** Nitrobenzene exposure has been associated with histopathology changes in the nasal cavity of rats (hyperplasia, inflammatory changes, suppurative exudate) and mice (olfactory epithelial degeneration, glandularization of respiratory epithelium) and in the lungs (alveolar/ bronchiolar hyperplasia and alveolar bronchiolization) of mice exposed by inhalation for intermediate and chronic durations.

- ***Hepatic:*** The liver is a target for nitrobenzene toxicity as evidenced by experimental animal studies that reported increased liver weights, degenerative changes in hepatocytes, hepatocytomegaly, and centrilobular necrosis. Some histopathology changes observed in the

livers of exposed animals (e.g., extramedullary hematopoiesis and hemosiderosis) are attributable to the hematologic effects of nitrobenzene.

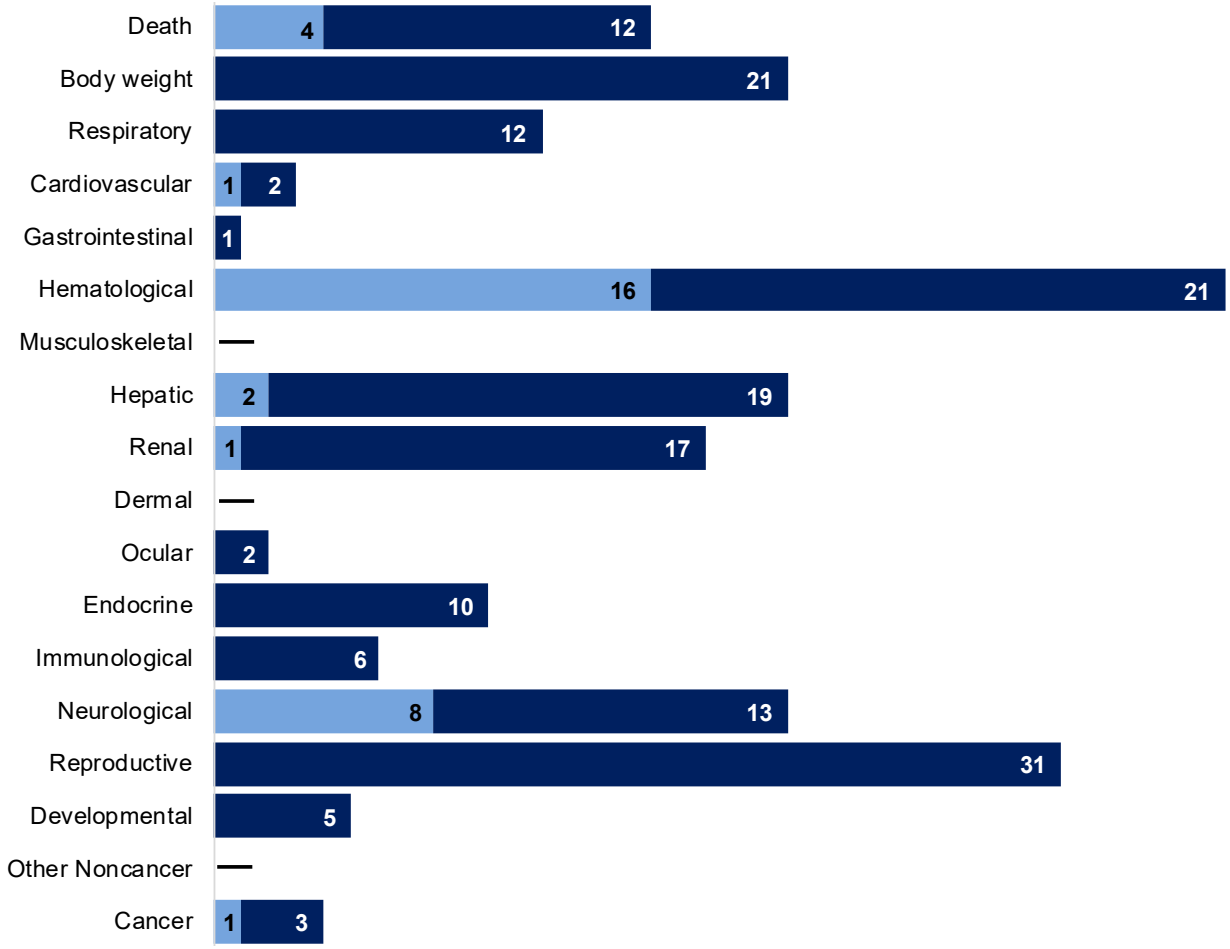
- ***Renal:*** Renal effects of nitrobenzene in animals include increases in kidney weight, increased incidence or severity of nephrosis, renal tubular hyperplasia, cysts, fibrosis, glomerular shrinkage, and degenerative changes in the cortical tubules.

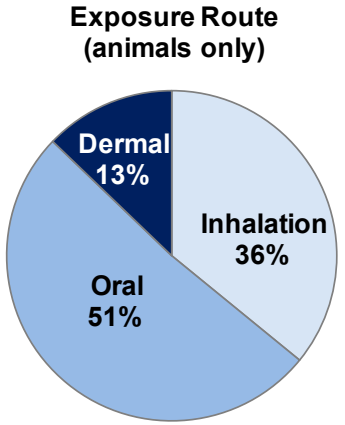
- ***Endocrine:*** Nitrobenzene effects on endocrine organs of laboratory rodents included increased incidences of adrenal cortical cell vacuolization and/or fatty changes in mice; increased basophilia of the adrenal medullary cells in rats; thyroid follicular cell hypertrophy, hyperplasia, and/or decreased serum thyroid-stimulating hormone (TSH) in rats and mice; and diffuse hyperplasia of the parathyroid glands in rats.

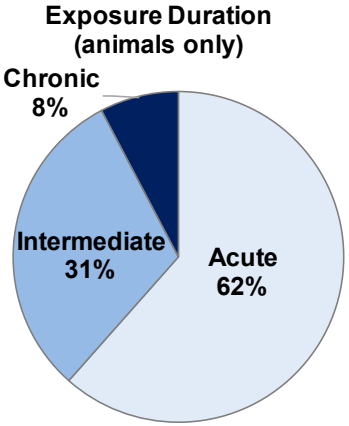
- ***Reproductive:*** Nitrobenzene is a known male reproductive toxicant and is often used as a positive control in studies of testicular toxicity. Rats and mice exposed to nitrobenzene via inhalation, oral, and dermal routes have exhibited decreases in testes and/or epididymal weights; atrophy of the testes and seminiferous tubules; hypospermatogenesis; Leydig cell hyperplasia; and dysfunctional spermiogenesis. Inhalation and oral studies have also shown that the effects on the male reproductive organs were associated with decreased fertility indices.

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

Most studies examined the potential reproductive, body weight, hematology, hepatic, renal, and neurological effects of nitrobenzene.
Fewer studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)







*Includes studies discussed in Chapter 2. A total of 68 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
1	Rat (F344) 10 M, 10 F	14 days, 5 days/week, 6 hours/day (WB)	0, 9.1, 35.8, 124.5	LE, CS, BC, HE, OW, GN, HP	Resp	124.5			
					Hemato		9.1		Splenic lesions (not specified); increased relative spleen weight (33%); at 35.8 ppm, effects included sinusoidal congestion, hemosiderosis, extramedullary hematopoiesis in spleen; and markedly increased relative spleen weight (89–111%)
					Hepatic	9.1 F	9.1 M 35.8 F		Increased relative liver weight (13% in males and 27% in females)
					Renal	9.1F	9.1 M 35.8 F	124.5	LOAEL: Increased relative kidney weight (15% in males and 23% in females) SLOAEL: Severe hyaline nephrosis
					Neuro	124.5			
					Repro	35.8 M		124.5 M	Severe dysfunctional spermiogenesis; increased multinucleated giant cells and interstitial edema in testes; Sertoli cell hyperplasia; 44% reduction in testes weight

Medinsky and Irons 1985

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
2	Rat (CD) 10 M, 10 F	5–14 days, 5 days/week, 6 hours/day (WB)	0, 9.1, 35.8, 124.5	LE, CS, BC, HE, OW, GN, HP	Death			124.5	5/10 male and 3/10 females found dead on 4th exposure day; remainder sacrificed moribund at end of week 1
					Resp		124.5		Perivascular edema and vascular congestion in lungs
					Hemato		9.1[b]		Decreased RBC count; increased relative spleen weight (44% in females); splenic lesions (not specified); increased methemoglobin in females (6.3 versus 4.8% in controls) ($BMCL_{1SD}$ = 16.3 ppm). At 35.8 ppm, effects included sinusoidal congestion, hemosiderosis and extramedullary hematopoiesis (both sexes); lymphoid hypoplasia and stromal hyperplasia (males) of spleen; markedly increased relative spleen weights (74–94%); and increased methemoglobin in males (8.7 versus 6.9% in controls).
					Hepatic	9.1	35.8		Mild hepatocyte necrosis; at 124.5 ppm, effects included sinusoidal congestion, basophilic and centrilobular hepatocyte degeneration, and hepatocyte necrosis

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Renal		124.5		Moderate to severe hydropic degeneration of cortical tubular cells
					Neuro	35.8		124.5	Perivascular hemorrhage in cerebellar peduncle; edema and malacia
					Repro			124.5 M	Moderate dysfunctional spermiogenesis
Medinsky and Irons 1985									
3	Rat (CD) 26 F	GDs 6–15, 6 hours/day (WB)	0, 1.06, 9.8, 39.4	LE, CS, BW, OW, GN, DX	Bd wt	9.8	39.4		Decreased maternal weight gain (19% compared to control during exposure)
					Hemato	1.06	9.8		Increased absolute and relative maternal spleen weights (13–15%)
					Develop	9.8	39.4		Increased incidences of litters with variations (hole in parietal bone and ecchymosis on trunk)
Tyl et al. 1987									
4	Mouse (B6C3F1) 10 M, 10 F	2-14 days, 5 days/week, 6 hours/day (WB)	0, 9.1, 35.8, 124.5	LE, CS, BC, HE, OW, GN, HP	Death			124.5	All mice were prostrate with bradypnea and dyspnea, and therefore sacrificed humanely between days 2 and 4 of exposure
					Resp		35.8		Mild bronchiolar hyperplasia
					Hemato		9.1		Splenic lesions (not specified); at 35.8 ppm effects included splenic sinusoidal congestion, extramedullary hematopoiesis, hemosiderosis, lymphoid hypoplasia, and stromal hypoplasia

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Hepatic		124.5 M		Centrilobular necrosis and severe hydropic degeneration
					Neuro	35.8		124.5	Perivascular hemorrhage in the cerebellar peduncle
					Repro		35.8 M	124.5 M	LOAEL: Testicular degeneration and increased multinucleated giant cells SLOAEL: Absence of spermatozoa in seminiferous tubules; degeneration of tubular epithelial cells; spermatocyte maturation arrest
Medinsky and Irons 1985									
5	Rabbit (New Zealand) 12 F	GDs 7–19, 6 hours/day (WB)	0, 10, 40, 81	LE, CS, BW, HE, OW, GN, DX	Bd wt	81			
					Hemato	10	40		Increased maternal methemoglobin (1.7 versus 1.0% in controls)
					Develop	81			No effect on numbers of corpora lutea, implantations, resorptions, or fetuses
Biodynamics 1983									
6	Rabbit (New Zealand) 22 F	GDs 7–19, 6 hours/day (WB)	0, 9.9, 41, 104	LE, CS, BW, HE, OW, GN, DX	Bd wt	41		104	Maternal body weight loss during gestation (10 g loss from GD 7 to GD 19 versus gain of 34 g in controls)
					Hemato	9.9	41		Increased maternal methemoglobin (1.4 versus 1.0% in controls)
					Develop	9.9	41		Decreased mean number viable male fetuses

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Biodynamics 1984									
INTERMEDIATE EXPOSURE									
7	Rat (CD) 120 M, 120 F	10 weeks (2 generations), 5 days/week, 6 hours/day (WB)	0, 1, 10, 40	BW, LE, RX, GN, OW	Repro	10		40	Decreased fertility rate; atrophy of seminiferous tubules; spermatocyte degeneration; reduced testicular and epididymal weights
					Develop	10	40		12% decrease in mean body weight of F1 offspring on PND 21
Dodd et al. 1987									
8	Rat (Fischer-344) 10 M, 10 F	90 days, 5 days/week, 6 hours/day (WB)	0, 5, 15.8, 48.7	CS, HP, BW, BC, HE, GN, UR, OW, BI	Bd wt	48.7			
					Resp	15.8 M 48.7 F	48.7 M		Minimal to slight hyperplasia of the bronchial epithelium in males
					Gastro	48.7 M	5 F		Diarrhea in females
					Hemato		5[c]		

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
									Increased methemoglobin in males (3.0 versus 1.2% in controls); hematology changes indicative of hemolytic anemia in females; minimal to slight acute sinusoidal congestion and moderate to marked hemosiderin deposition in spleen in both sexes. At 48.7 ppm, effects included moderate sinusoidal congestion, proliferation of mesenchymal cells, fibroblastic hyperplasia of the splenic capsule, accumulation of lymphocytes and macrophages, and extramedullary hematopoiesis in spleen; and bone marrow hyperplasia.
					Hepatic		15.8	48.7	LOAEL: Increased liver weights SLOAEL: Disorganized hepatic cords, vascular ectasia, centrilobular hepatocyte degeneration, and periportal hepatocyte basophilia (males), focal necrosis (females)
					Renal	15.8 F	5 M 48.7 F		Minimal nephrosis in males, slight to moderate nephrosis in females
					Endocr	15.8	48.7		Increased basophilia of medullary cells in adrenal glands

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Repro	15.8 M		48.7 M	Moderate to severe degeneration of tubular epithelial cells, absence of mature sperm in epididymis; slight to moderate interstitial edema in testes; minimal to slight interstitial cell hyperplasia in testes; 33% decrease in testes weight
Hamm et al. 1984									
9	Rat (CD) 10 M, 10 F	90 days, 5 days/week, 6 hours/day (WB)	0, 5, 15.8, 48.7	CS, HP, BW, BC, HE, GN, UR, OW, BI	Bd wt	48.7			
					Resp	15.8 M 48.7 F	48.7 M		Epithelial hyperplasia/metaplasia and goblet cell hyperplasia in nasal turbinates of males
					Hemato		5		Moderate hemosiderin pigmentation (males) and slight to moderate sinusoidal congestion (both sexes) in spleen
					Hepatic	5 F	5 M 15.8 F		Microgranulomas in males; centrilobular hepatocyte hypertrophy, increased liver weight, and 4-fold increase in serum ALT in females
					Renal	5 M 48.7 F	15.8 M	48.7 M	LOAEL: Minimal to slight toxic nephrosis in males SLOAEL: Moderate to marked toxic nephrosis in males
					Endocr	15.8 M 48.7 F	48.7 M		Basophilia of adrenal medullary cells and slight thyroid follicular cell hypertrophy in males

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Repro	5 M	15.8 M	48.7 M	LOAEL: slight reduction in mature sperm in two animals SLOAEL: Bilateral testicular atrophy, complete loss of seminiferous epithelium, and absence of mature sperm in lumen of epididymis in all animals; increased interstitial cell hyperplasia, interstitial testicular atrophy, and multinucleate giant cells; 55% reduction in testes weight
Hamm et al. 1984									
10	Mouse (B6C3F1) 10 M, 10 F	90 days, 5 days/week, 6 hours/day (WB)	0, 5, 15.8, 48.7	CS, BW, BC, HE, UR, OW, GN, HP	Bd wt	48.7			
					Resp	15.8	48.7		Minimal to slight hyperplasia of the bronchial mucosa
					Hemato		5		Minimal to slight hemosiderin deposition (both sexes) and slight to moderate sinusoidal congestion (females) in spleen; at 48.7 ppm, effects included 45–66% increase in spleen weights and bone marrow hyperplasia
					Hepatic	15.8	48.7		Centrilobular hepatocyte hyperplasia with some cord disorganization (females) and basophilic hepatocytes (males); increased liver weight; increased ALT (males)
					Renal	48.7			
					Endocr	48.7 M	5 F	48.7 F	

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
									LOAEL: Minimal to slight cortical cell vacuolization in zona reticularis of adrenal glands in females SLOAEL: Marked to very severe cortical cell vacuolization in adrenal glands of females
					Repro	48.7			No significant histopathology changes in reproductive organs
Hamm et al. 1984									
CHRONIC EXPOSURE									
11	Rat (Fischer-344) 70 M, 70 F	2 years, 5 days/week, 6 hours/day (WB)	0, 1, 5, 24.8	LE, CS, BW, HE, GN, HP	Bd wt	24.8			
					Resp		1		Increased pigmented olfactory epithelium in nose
					Gastro	5 M 24.8 F	24.8 M		Increased focal pancreatic acinar cell hyperplasia in males
					Hemato		1		Increased spleen congestion and pigmentation in the spleen
					Hepatic	1 M 5 F	5 M 24.8 F		Increased eosinophilic foci and centrilobular hepatocytomegaly (males) and eosinophilic foci and spongiosis hepatis (females)
					Renal	5 M 24.8 F	24.8 M		Increased renal tubular hyperplasia in males
					Endocr	5 M 24.8 F	24.8 M		Increased diffuse hyperplasia of the parathyroid glands in males
					Repro	24.8			

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Cancer			24.8	CEL: Increased incidences of endometrial stromal polyps in the uterus (females), hepatocellular adenoma or carcinoma (males), and renal tubular adenoma or carcinoma (males)
Cattley et al. 1994, 1995; CIIT 1993									
12	Rat (CD) 70 M	2 years, 5 days/week, 6 hours/day (WB)	0, 1, 5, 24.8	LE, CS, BW, HE, GN, HP	Bd wt	24.8			
					Resp		1[d]		Increased squamous epithelial hyperplasia in nose
					Hemato		1		Increased methemoglobin at 15-month sacrifice (4.08 versus 1.18% in controls); increased splenic congestion
					Hepatic		1		Increased Kupffer cell pigmentation
					Renal	24.8			
					Endocr	24.8			
					Repro	5		24.8	Increased bilateral atrophy of the testes and bilateral hypospermia in the epididymis
					Cancer			24.8	CEL: Increased incidences of hepatocellular adenoma and adenoma or carcinoma
Cattley et al. 1994, 1995; CIIT 1993									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
13	Mouse (B6C3F1) 70 M, 70 F	2 years, 5 days/week, 6 hours/day (WB)	0, 5, 24.8, 49.1	LE, CS, BW, HE, GN, HP	Bd wt	49.1			
					Resp		5		Bronchiolization of the alveoli and pigment deposition in olfactory epithelium (both sexes); degeneration of nasal olfactory epithelium in females
					Hemato	5 F 24.8 M	24.8 F 49.1 M		Increased methemoglobin in males (3.97 versus 1.97% in controls) and females (2.22 versus 1.39% in controls); increased bone marrow hypercellularity (males)
					Hepatic	24.8 F	5 M 49.1 F		Increased centrilobular hepatocytomegaly and multinucleated hepatocytes in males and centrilobular hepatocytomegaly in females
					Renal	49.1 F 24.8 M	49.1 M		Increased kidney cysts in males
					Endocr	5	24.8		Increased thyroid follicular cell hyperplasia in males; increased adrenal gland cortical cell vacuolization in females
					Immuno	24.8 F 49.1 M	49.1 F		Increased thymic involution in females
					Repro	24.8 M		49.1 M	Increased hypospermia of the epididymis

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Cancer			5 M 49.1 F	CEL: lung adenoma or carcinoma (males); mammary gland adenocarcinoma (females)

Cattley et al. 1994, 1995; CIIT 1993

[a]The number corresponds to the entries in Figure 2-2; differences in levels of health effects and cancer 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 an acute-duration inhalation MRL using benchmark dose analysis. The $BMCL_{1SD}$ of 16.3 ppm was adjusted to continuous exposure and converted to a $BMCL_{HEC}$ of 2.91 ppm. The $BMCL_{HEC}$ was divided by an uncertainty factor of 30 (10 for human variability and 3 for animal to human extrapolation after dosimetric adjustment), resulting in an MRL of 0.1 ppm.
[c]Used to derive an intermediate-duration inhalation MRL. The LOAEL of 5 ppm was adjusted to continuous exposure and converted to a $LOAEL_{HEC}$ of 0.89 ppm. The $LOAEL_{HEC}$ was divided by an uncertainty factor of 300 (10 for human variability, 3 for animal to human extrapolation after dosimetric adjustment, and 10 for use of a LOAEL), resulting in an MRL of 0.003 ppm.
[d]Used to derive a chronic-duration inhalation MRL. The LOAEL of 1 ppm was adjusted to continuous exposure and converted to a $LOAEL_{HEC}$ of 0.054 ppm. The $LOAEL_{HEC}$ was divided by an uncertainty factor of 300 (10 for human variability, 3 for animal to human extrapolation after dosimetric adjustment, and 10 for use of a LOAEL), resulting in an MRL of 0.0002 ppm.

ALT = alanine aminotransferase; BI = biochemical changes; BC = blood chemistry; Bd wt or BW = body weight; BMCL = benchmark concentration lower confidence limit; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; F = female(s); GN = gross necropsy; HE = hematology; HEC = human equivalent concentration; Hemato = hematological; HP = histopathological; Immuno = immunological; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; Neuro = neurological; NOAEL = no-observed-adverse-effect level; OW = organ weight; PND = postnatal day; RBC = red blood cell; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; SD = standard deviation; SLOAEL = serious LOAEL; UR = urinalysis; (WB) = whole body

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

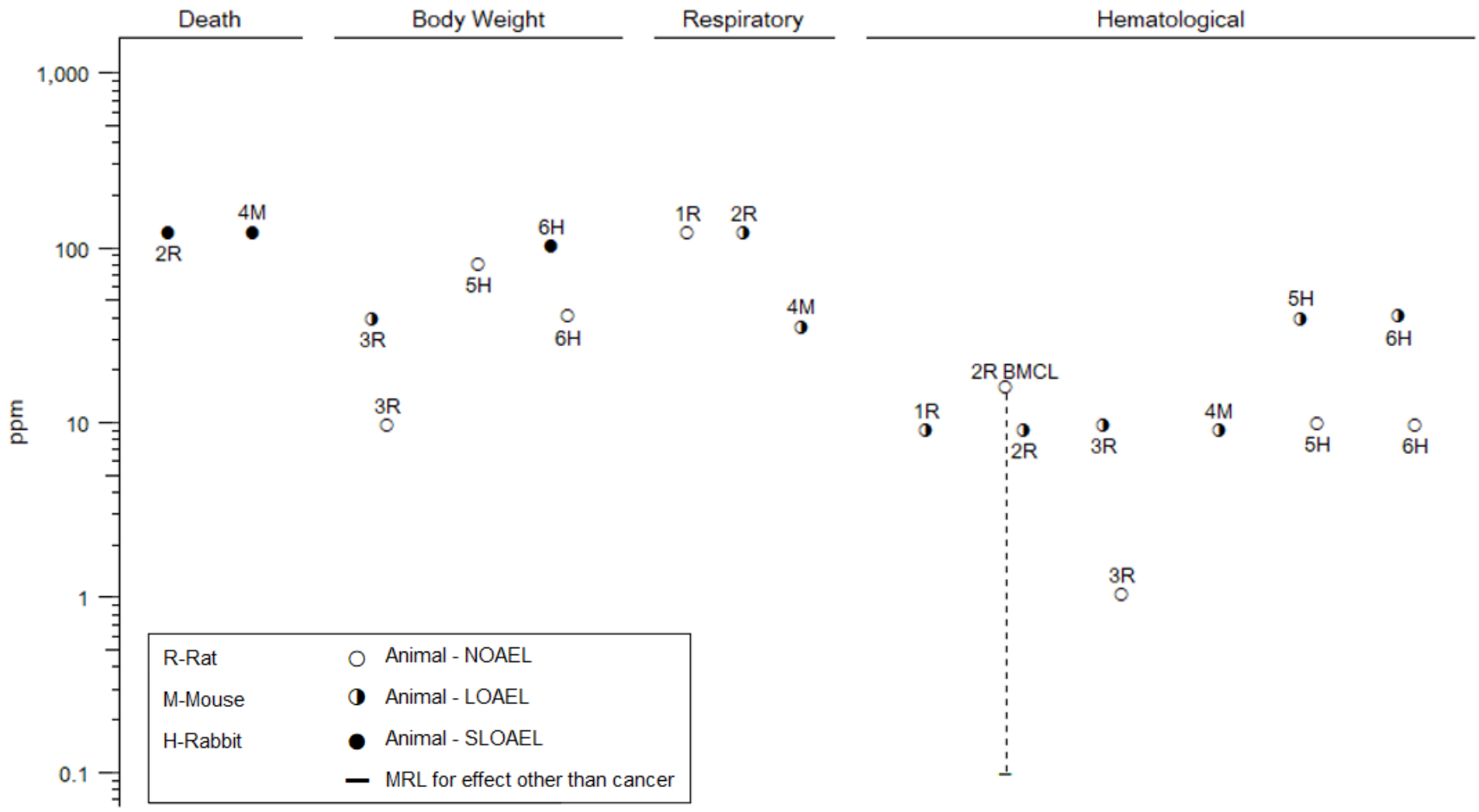

Figure 2-2. Levels of Significant Exposure to Nitrobenzene – Inhalation
Acute (≤14 days)

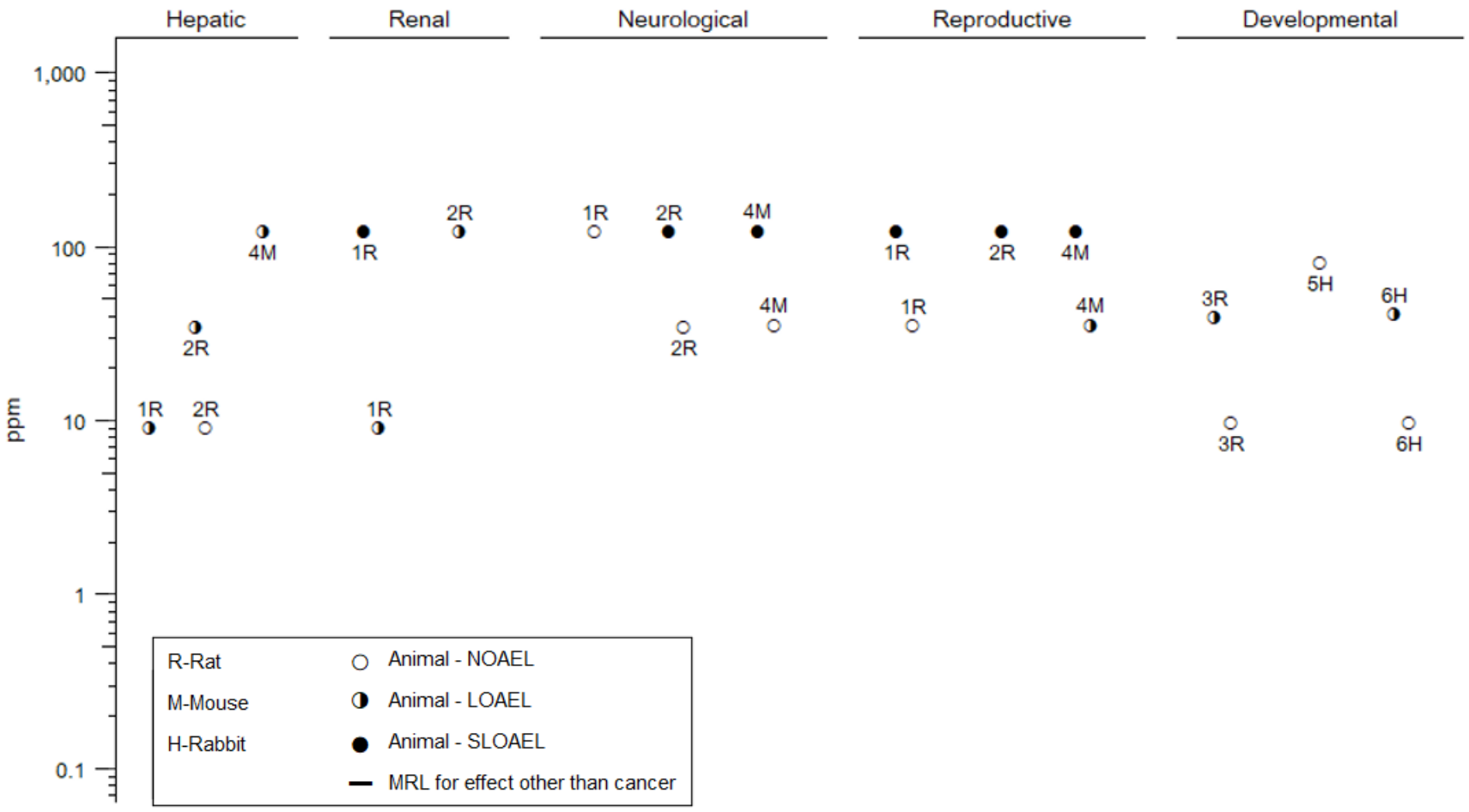

Figure 2-2. Levels of Significant Exposure to Nitrobenzene – Inhalation
Intermediate (15-364 days)

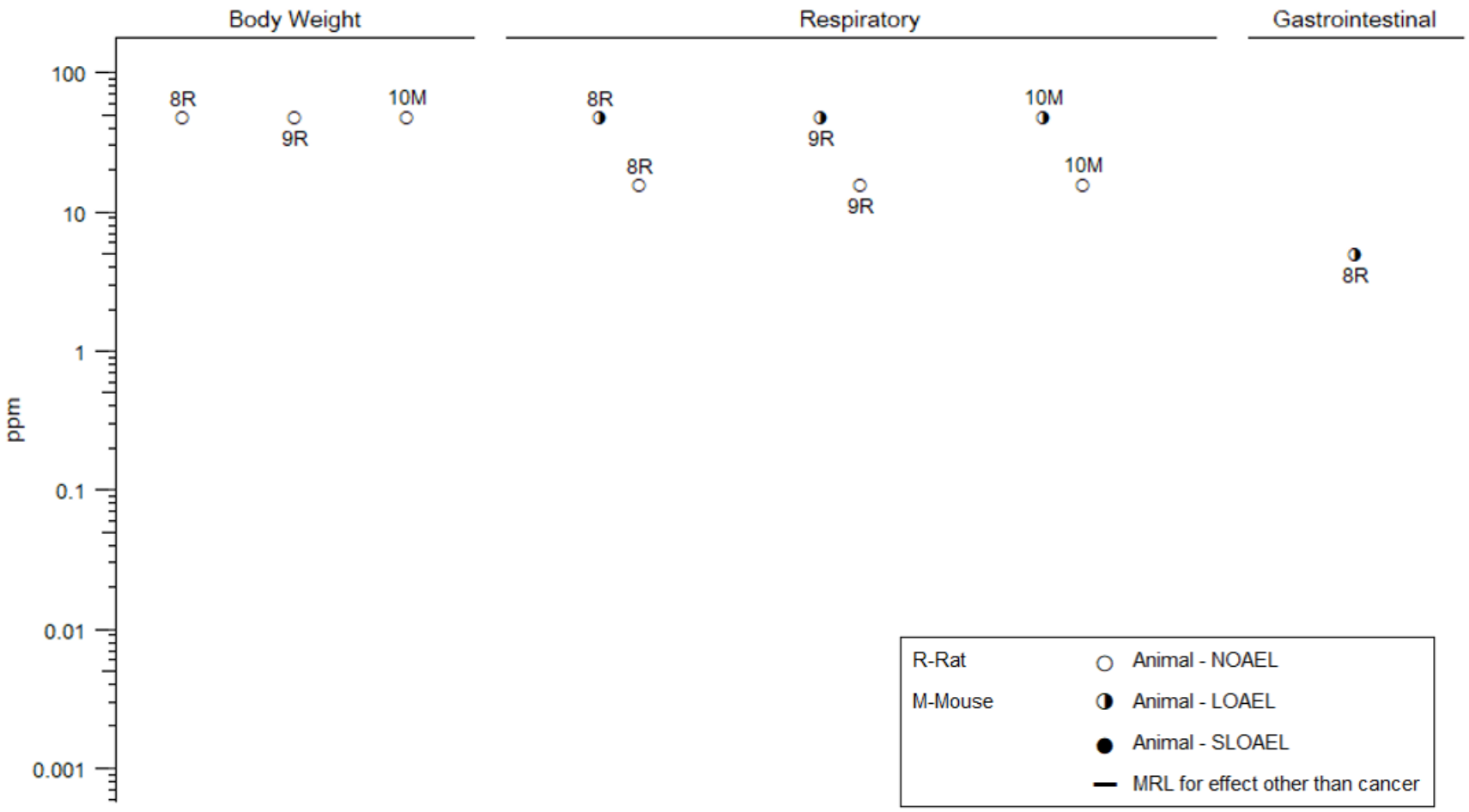

Figure 2-2. Levels of Significant Exposure to Nitrobenzene – Inhalation
Intermediate (15-364 days)

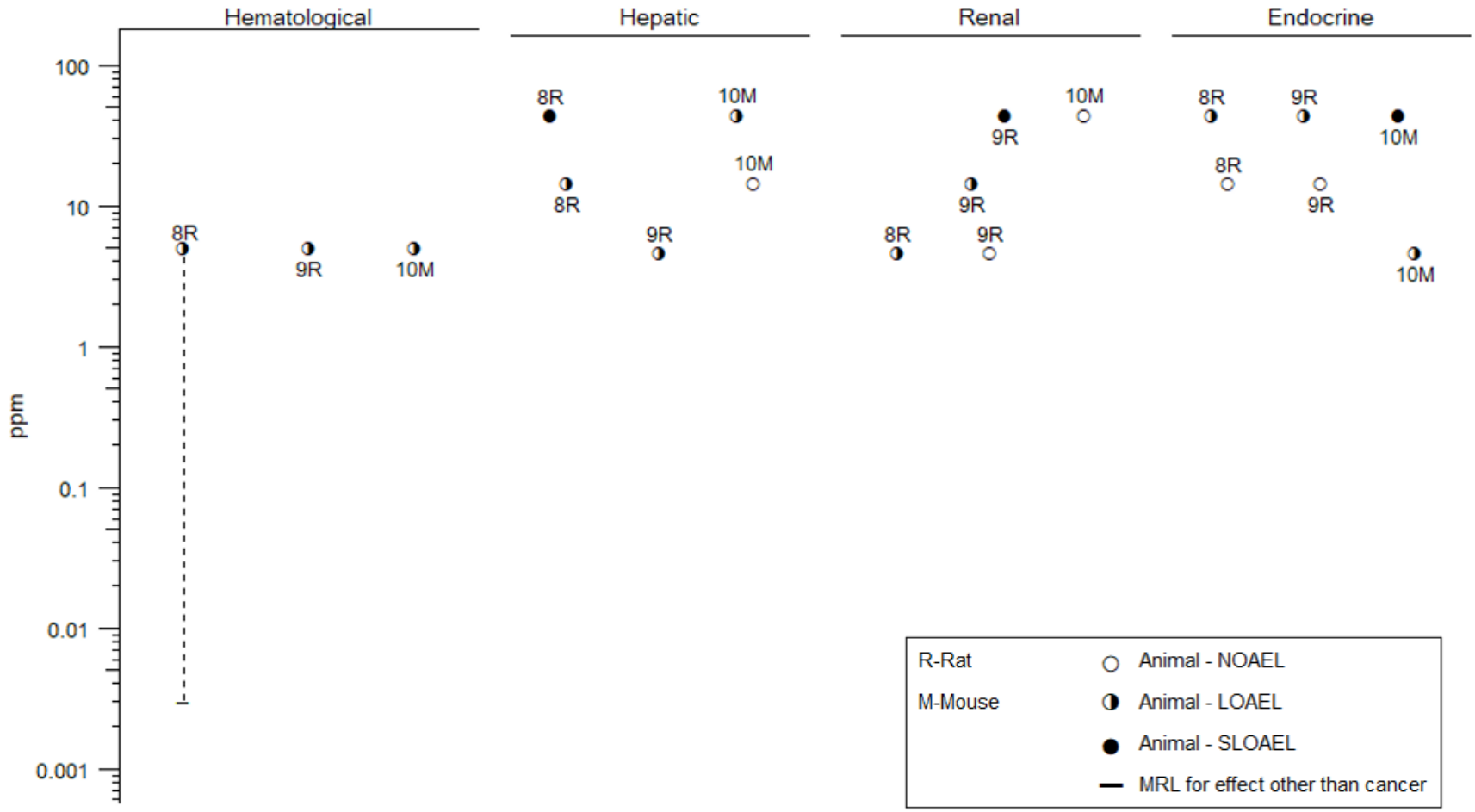

Figure 2-2. Levels of Significant Exposure to Nitrobenzene – Inhalation
Intermediate (15-364 days)

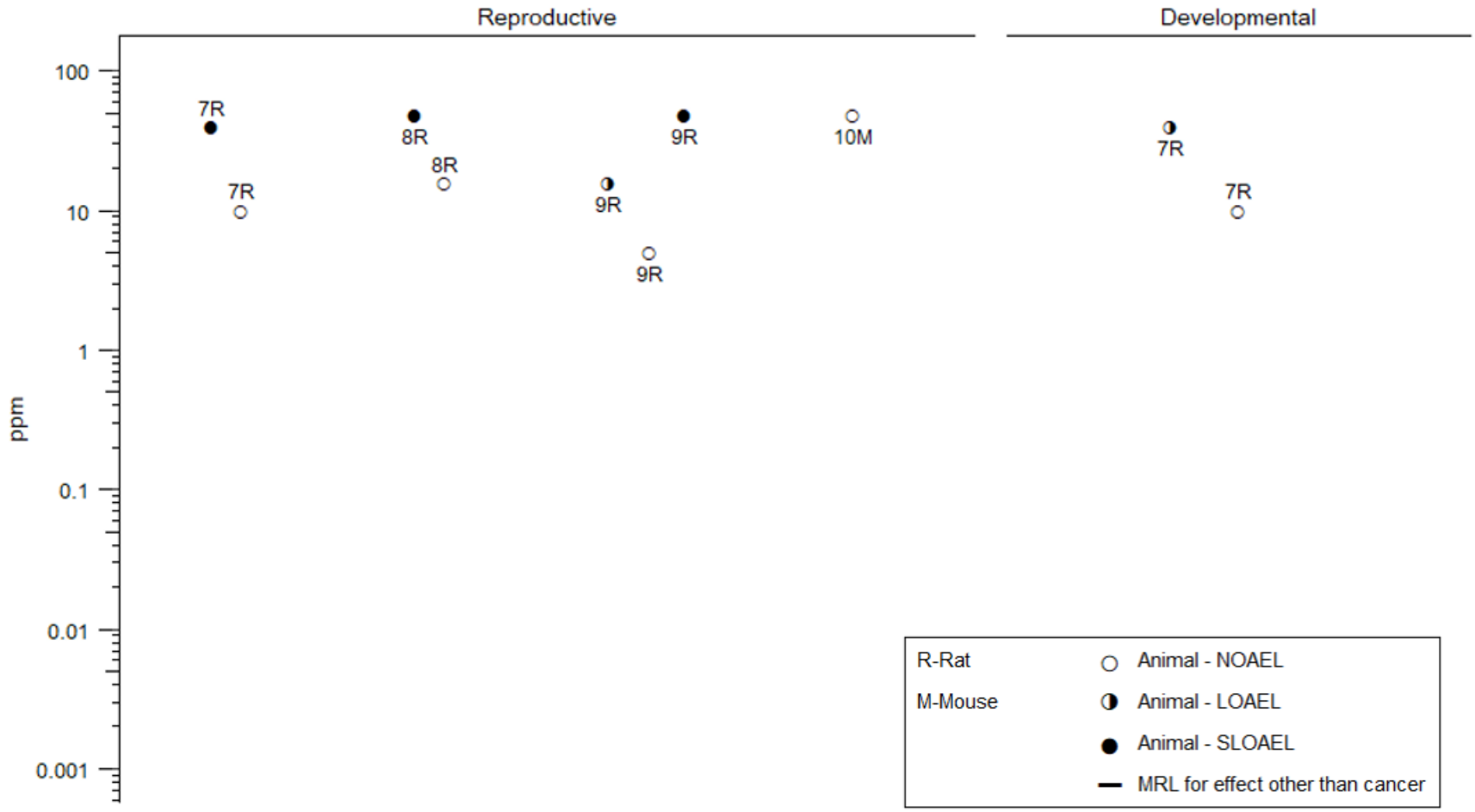

Figure 2-2. Levels of Significant Exposure to Nitrobenzene – Inhalation
Chronic (≥365 days)

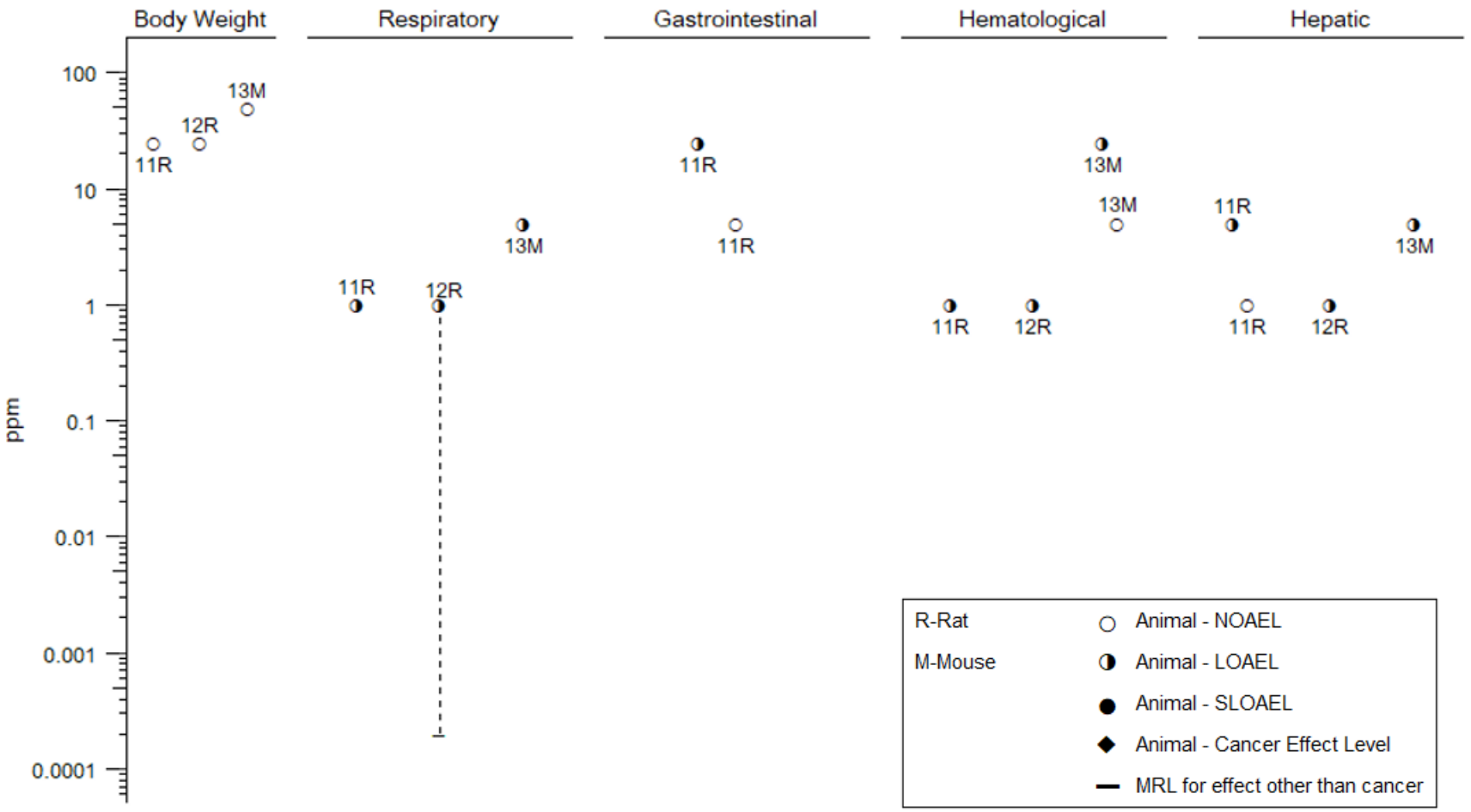

Figure 2-2. Levels of Significant Exposure to Nitrobenzene – Inhalation
Chronic (≥365 days)

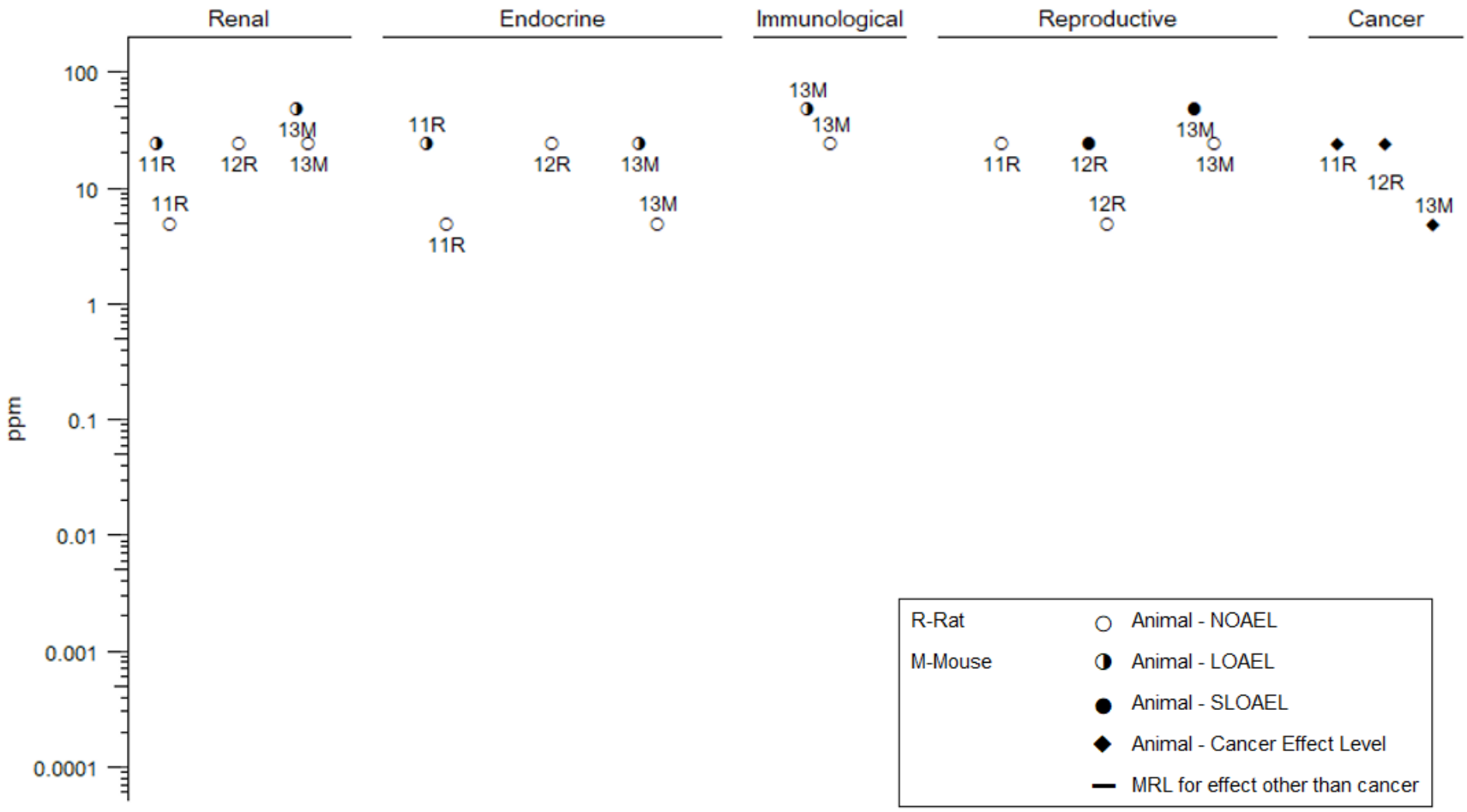

Table 2-2. Levels of Significant Exposure to Nitrobenzene – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL)	Effects
ACUTE EXPOSURE									
1	Rat (Sprague-Dawley) 5 M	2 weeks, daily (GO)	0, 60	OW	Repro		60		Decreased relative testes weight
Iida et al. 1997									
2	Rat (Sprague-Dawley) 8 M	3 days, daily (G)	0, 60	CS, BW, OW, HP	Bd wt Repro	60 60			
Kawaguchi et al. 2004									
3	Rat (Sprague-Dawley) 10 M	14 days, daily (GO)	0, 60	RX, OW	Repro		60		Significantly decreased testes and epididymal weights, 34% decrease in sperm count, and significant decrease in sperm motility with no effect on copulation or fertility rate
Kawashima et al. 1995									
4	Rat (Fischer-344) 45 M	Once (GO)	0, 300	HP	Repro			300	Marked degeneration of the seminiferous epithelium with presence of multinucleated giant cells and loss of mature spermatids
Levin et al. 1988									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL)	Effects
5	Rat (Sprague-Dawley) 6 M	Once (GO)	0, 300	OW, RX, HP	Repro			300	Degenerating and missing pachytene spermatocytes; immature germ cells and debris in epididymis; maturation depletion of spermatids; some multinucleated giant cells; decreased weights of testes and epididymis; decreased cauda and caput sperm counts and abnormal sperm morphology
Linder et al. 1992									
6	Rat (Wistar) 36 M	Once (GO)	0, 300	OW, RX, BI	Repro			300	13 and 23% decreases in testicular weight 1 and 3 days post-treatment, respectively
McLaren et al. 1993a									
7	Rat (Fischer-344) 10 M	Once (GO)	0, 550	CS, HP	Neuro			550	Moderate to severe ataxia, loss of righting reflex, unresponsive to stimuli; hemorrhages in the brain stem and cerebellum, bilateral symmetric degeneration in the cerebellum and cerebellar peduncles
Morgan et al. 1985									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL)	Effects
8	Rat (Wistar) 6 M	14 days, daily (G)	0, 100	BW, BC, BI, NX, OW, HP	Bd wt	100			
					Renal		100		Increased serum urea and creatinine; renal lesions including mild fibrosis and hemorrhage and marked glomerular shrinkage
					Neuro			100	Neurobehavioral changes (decreased exploratory behavior and increased defecation); increased acetylcholinesterase activity in brain; degenerative lesions in the cerebellum, cerebrum, and hippocampus
					Endocr		100		Decreased serum TSH
					Repro			100	Decreased testicular and epididymal weights; decreased serum prolactin, luteinizing hormone, follicle stimulating hormone, and testosterone; atrophic and degenerated seminiferous tubules
Oladele et al. 2020a, 2020b, 2021									
9	Rat (Sprague-Dawley) 3 M	Once (GO)	0, 250	HP	Repro			250	Degeneration of late pachytene spermatocytes; spermatid degeneration and formation of multinucleated giant cells; sloughing of cells into tubular lumen; loss of round and elongate spermatids
Shinoda et al. 1998									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL)	Effects
10	Mouse B6C3F1 8 F	14 days, Daily (GO)	0, 30, 100, 300	LE, CS, BW, BC, BI, HE, OW, GN, HP, IX	Death			300	8.5% of mice across several experiments died prematurely at this dose
					Bd wt	300			
					Resp		300		Increased lung weight
					Hemato		30[b]		Increased DNA synthesis, number of cells and number of granulocyte-monocyte progenitor cells in bone marrow ($BMDL_{1SD}$ = 4.7 mg/kg/day)
					Hepatic	30	100		Increased liver weight, hepatomegaly
					Renal	100	300		Increased kidney weight
					Immuno	30	100		Decreased IgM AFC in spleen cells; decreased natural killer cell activity
					Neuro			300	Ataxia, lethargy, and circling behavior
Burns et al. 1994									
INTERMEDIATE EXPOSURE									
11	Rat (Sprague-Dawley) 5 M	3 weeks, daily (GO)	0, 60	OW, HP	Repro			60	Decreased testes weight (50%); atrophy of the epididymis
Iida et al. 1997									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL)	Effects
12	Rat (Sprague-Dawley) 4 M	49 days, daily (GO)	0, 20, 40, 60	RX	Repro	20		40	Absence of motile sperm
Kato et al. 2002									
13	Rat (Sprague-Dawley) 8 M	18 days, daily (G)		CS, BW, OW, HP, RX	Bd wt Repro	60		60	Decreased weights of testes and epididymides; severe atrophy of seminiferous tubules; decrease in sperm concentration and cell debris in tubular lumina of caput/corpus and cauda epididymides; increased percent detached sperm heads and decreased percentage motile sperm, sperm velocities, and amplitudes of sperm heads
Kawaguchi et al. 2004									
14	Rat (Sprague-Dawley) 10 M	21–70 days, daily (GO)	0, 60	RX, OW	Repro			60	Fertility index decreased to <20% (0% after 28 days); significantly decreased testes weight (>50%) and epididymal weights; sperm count decreased to 10% of controls; decreased sperm motility and viability; and increased % abnormal sperm
Kawashima et al. 1995									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL)	Effects
15	Rat (Sprague-Dawley) 10 M, 10 F	54 days, daily (GO)	0, 20, 60, 100	LE, BW, HE, BC, RX, DX, GN, OW, HP	Death			100	2/10 males and 9/10 females died prior to study termination
					Bd wt	60 M	100 M		17% decrease in body weight of males
					Hemato		20 M		Decreased erythrocytes, hemoglobin, and packed cell volume; increased hematopoiesis in bone marrow; increased spleen weight; extramedullary hematopoiesis and hemosiderin deposition in the spleen in males
					Hepatic		20 M		Increased liver weights; centrilobular swelling of hepatocytes, hemosiderin deposition in Kupffer cells, extramedullary hematopoiesis in males
					Renal		20 M		Hemosiderin deposition in proximal tubules in males
					Neuro	20		60	LOAEL: Necrosis/gliosis in cerebellar medulla and pons (3/10 males); torticollis and abnormal gait (females)
					Repro	20 M		60 M	~60% decrease in testes weight and ~20% decrease in epididymides weight; atrophy of seminiferous tubules (all males), Leydig cell hyperplasia, loss of intraluminal sperm or cell debris in epididymis
					Develop		20	60	

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL)	Effects
									LOAEL: decreased male pup weight on PND 4 (6% relative to controls) SLOAEL: decreased pup viability and >20% reduction in pup weights on PND 4
Mitsumori et al. 1994									
16	Rat (Fischer-344) 10 M, 10 F	90 days daily (GO)	0, 9.375, 18.75, 37.5, 75, 150	LE, CS, BW, FI, HE, GN, OW, HP	Death			150	9/10 males and 3/10 females died between weeks 6 and 13
					Bd wt	75 M 150 F		150 M	30% lower final body weight in the surviving male
					Resp	37.5 F 75 M	75 F		Increased lung weight in females
					Cardio	75 M	9.375 F		Increased heart weight in females
					Hemato		9.375[c]	75	LOAEL: increased absolute reticulocyte count and decreased hemoglobin; increased methemoglobin in males (2.752 versus 1.131% in controls) and females (2.059 versus 0.941% in controls) ($BMDL_{1SD}$ = 1.8 mg/kg/day) SLOAEL: cyanosis
					Hepatic		9.375		Increased liver weights
					Renal	37.5 F	9.375 M 75 F		Increased kidney weights (both sexes); pigment deposition in kidney (females)
					Immuno	9.375		18.75 F	Increased lymphoid depletion in spleen
					Neuro			75	Ataxia (females); hemorrhage in brainstem (males)

Table 2-2. Levels of Significant Exposure to Nitrobenzene – 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	18.75 M	37.5 M 150 F	75 M	LOAEL: Atrophic seminiferous tubules in one male; atrophied uteri in 2 females SLOAEL: atrophy of the testes in 9/10 males; hypospermatogenesis; ~40% decreased testes weight
NTP 1983a									
17	Mouse (B6C3F1) 10 M, 10 F	90 days, daily (GO)	0, 18.75, 37.5, 75, 150, 300	LE, CS, BW, FI, HE, GN, OW, HP	Death			300 M	3/10 males died between weeks 4 and 5; 2/10 males were sacrificed moribund
					Bd wt	150			
					Hemato		18.75		Increased methemoglobin in males (2.162 versus 1.074% in controls) and females (1.198 versus 0.871% in controls); increased reticulocytes in females
					Hepatic	75 M	18.75 F 150 M		Increased liver weights
					Renal	150 M 300 F	300 M		Increased kidney weight in males
					Endocr	150 F	300 F		Fatty change in zona reticularis of adrenal glands of females
					Immuno			150 F	Lymphoid depletion in females

Table 2-2. Levels of Significant Exposure to Nitrobenzene – 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	37.5 M 75 F	75 M	300	LOAEL: Lethargy in 1/10 males SLOAEL: Ataxia, lethargy (males); irritability, ataxia, and head bobbing behavior (females)
					Repro	75 M		150 M	Testicular atrophy
NTP 1983a									

[a]The number corresponds to the entries in Figure 2-3; differences in levels of health effects and cancer between male and females are not indicated in Figure 2-3. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an acute-duration oral MRL. The data on increased DNA synthesis in bone marrow were subjected to benchmark dose modeling, resulting in a $BMDL_{1SD}$ of 4.7 mg/kg/day. The $BMDL_{1SD}$ was divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability), resulting in an MRL of 0.05 mg/kg/day.
[c]Used to derive an intermediate-duration oral MRL. The data on increased methemoglobin were subjected to BMD modeling, resulting in a $BMDL_{1SD}$ of 1.8 mg/kg/day. The $BMDL_{1SD}$ was divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability), resulting in a MRL of 0.02 mg/kg/day.

AFC = antibody forming cell; BI = biochemical changes; BC = blood chemistry; Bd wt or BW = body weight; BMD = benchmark dose; BMDL = lower confidence limit on the benchmark dose; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DNA = deoxyribonucleic acid; DX = developmental toxicity; Endocr = endocrine; F = female(s); FI = food intake; (G) = gavage; GN = gross necropsy; (GO) = gavage in oil vehicle; HE = hematology; Hemato = hematological; HP = histopathological; IgM = immunoglobin M; Immuno = immunological; IX = immune effects; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NX = neurological function; OW = organ weight; PND = postnatal day; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; SD = standard deviation; SLOAEL = serious LOAEL; TSH = thyroid-stimulating hormone

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

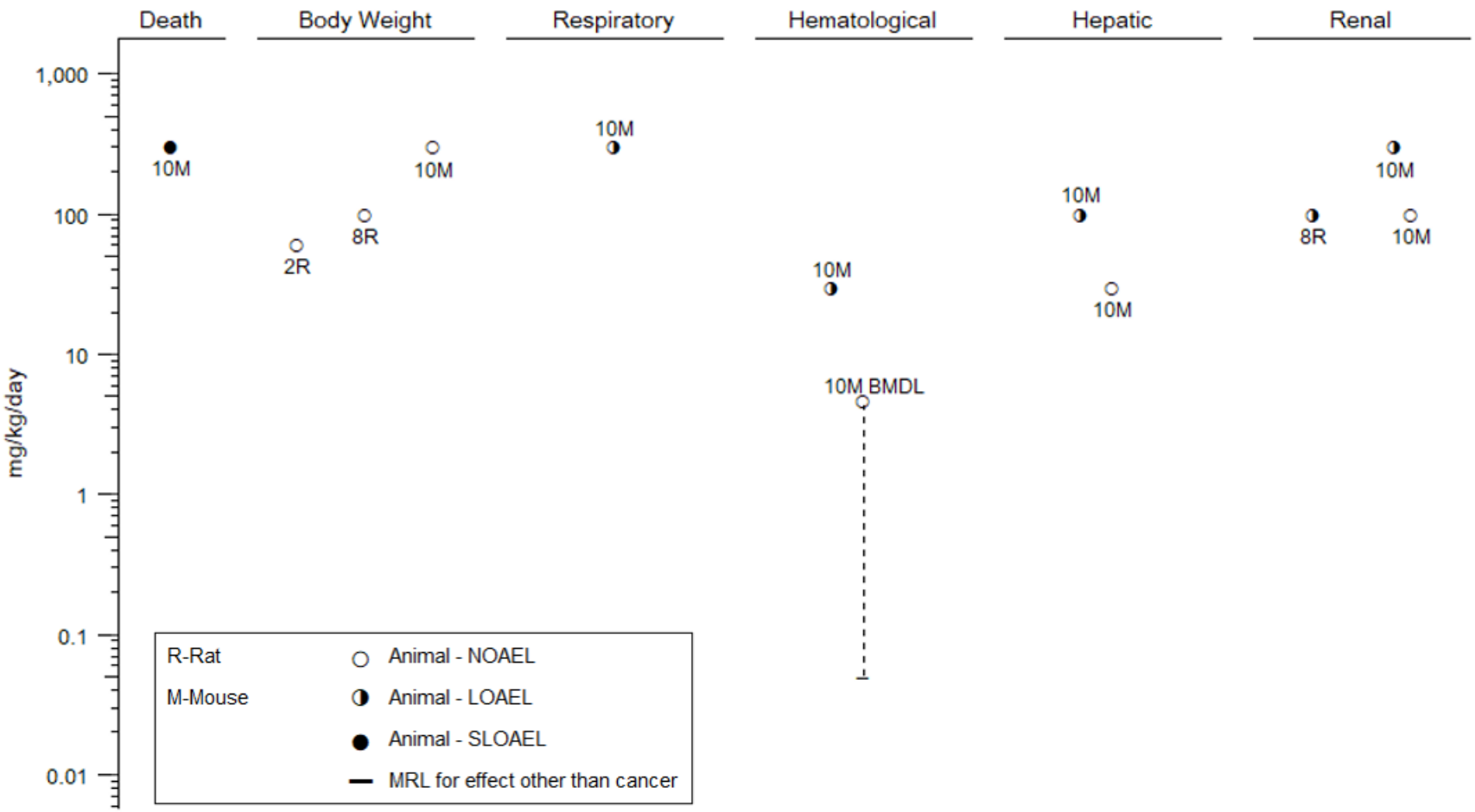

Figure 2-3. Levels of Significant Exposure to Nitrobenzene – Oral
Acute (≤14 days)

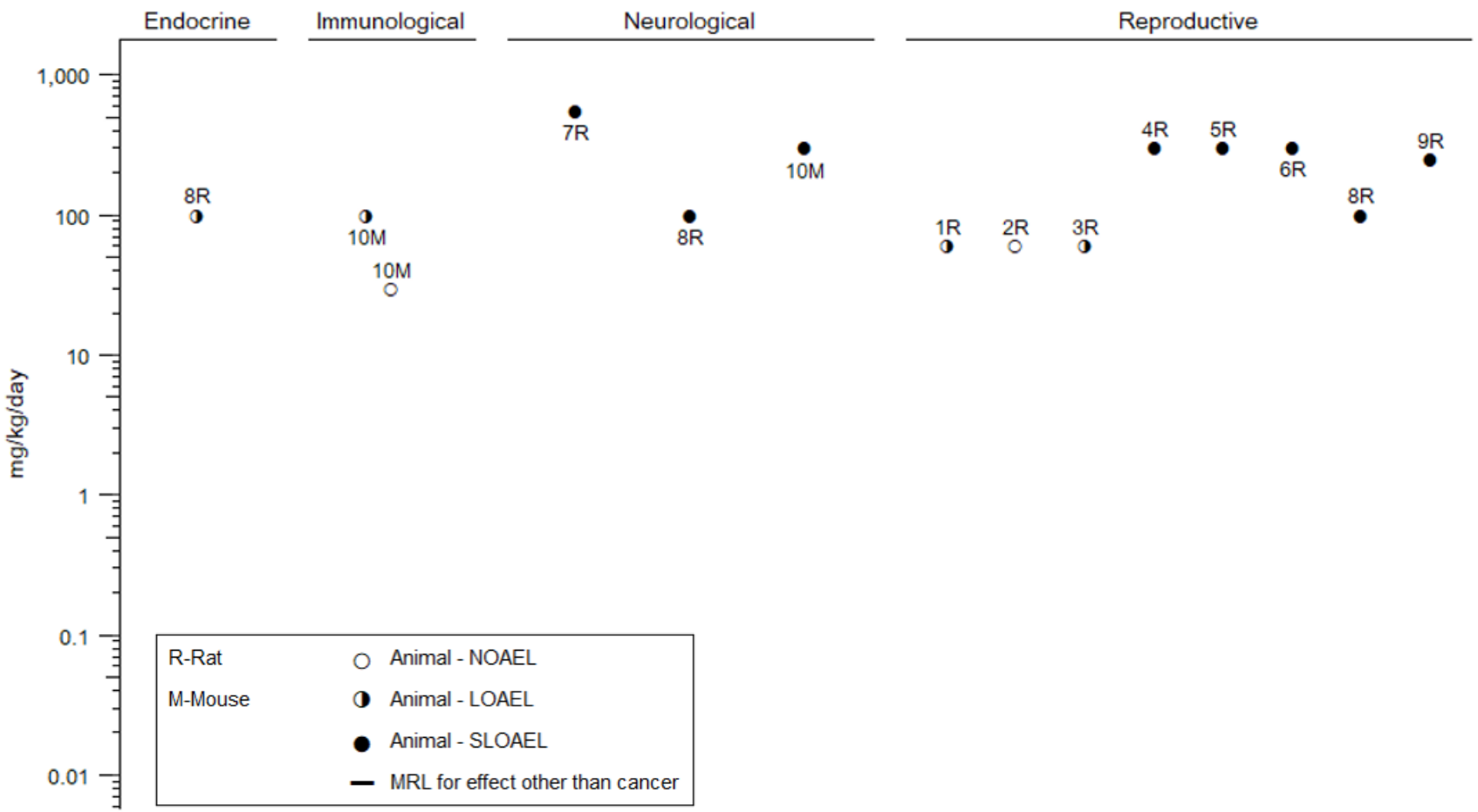

Figure 2-3. Levels of Significant Exposure to Nitrobenzene – Oral
Intermediate (15-364 days)

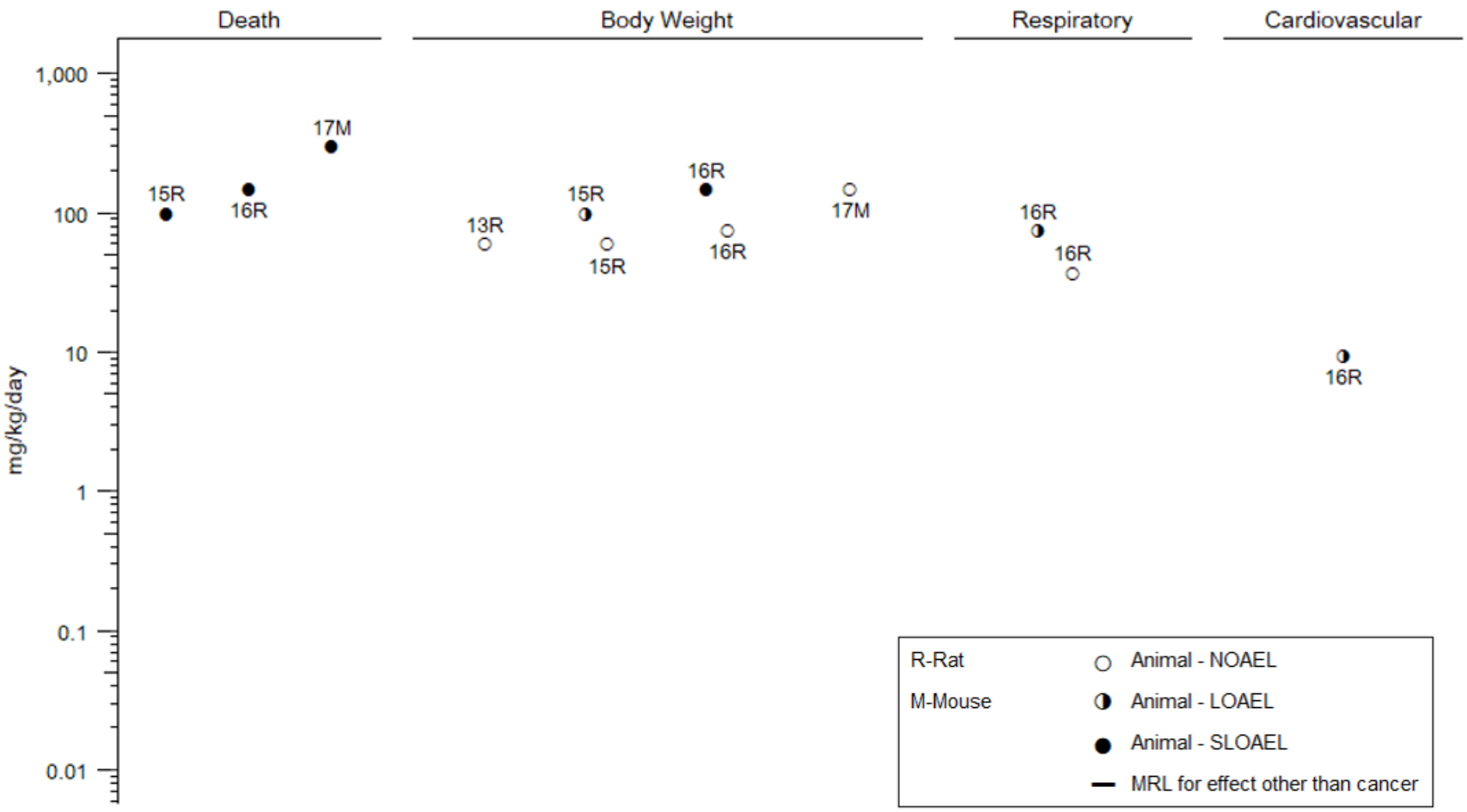

Figure 2-3. Levels of Significant Exposure to Nitrobenzene – Oral
Intermediate (15-364 days)

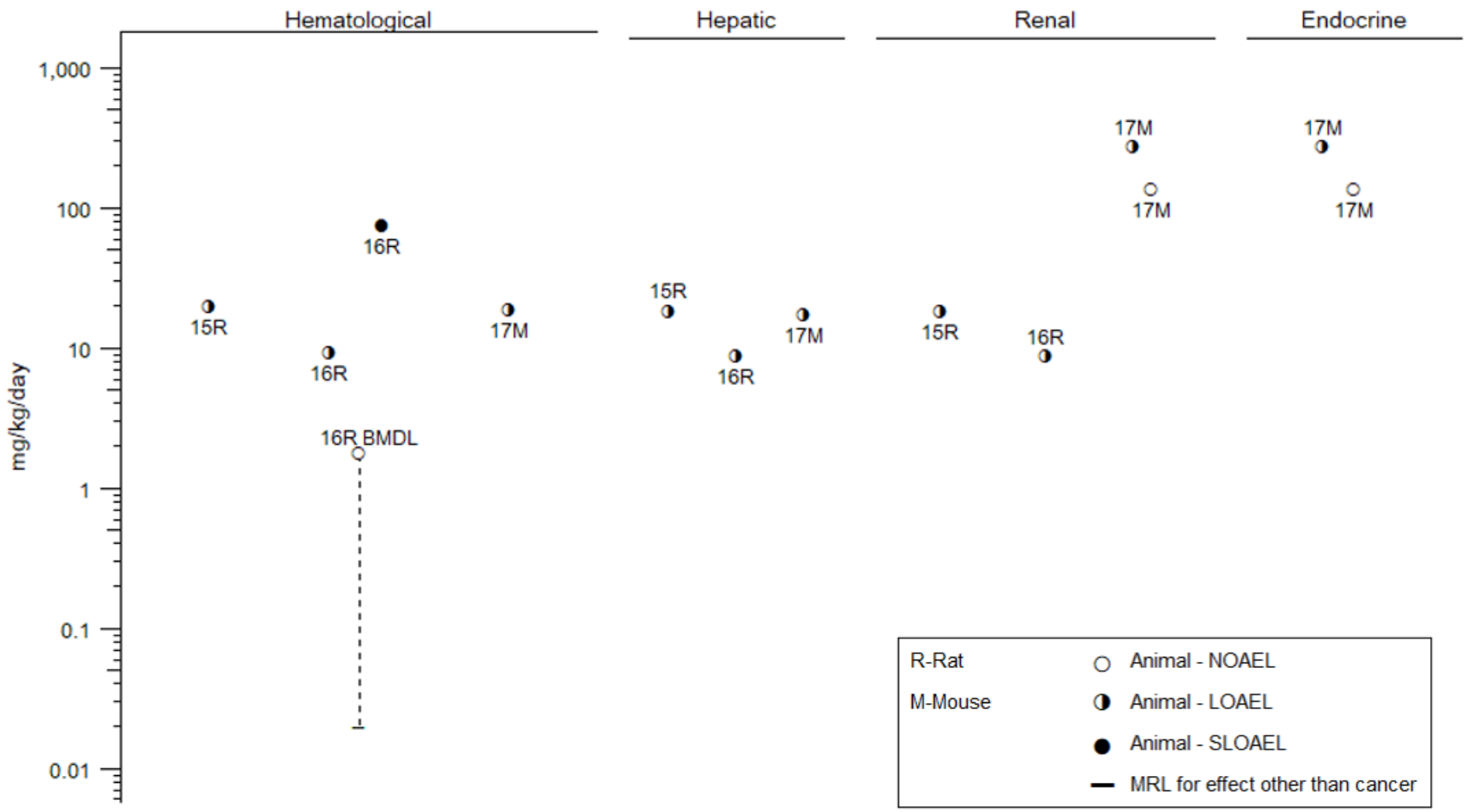

Figure 2-3. Levels of Significant Exposure to Nitrobenzene – Oral
Intermediate (15-364 days)

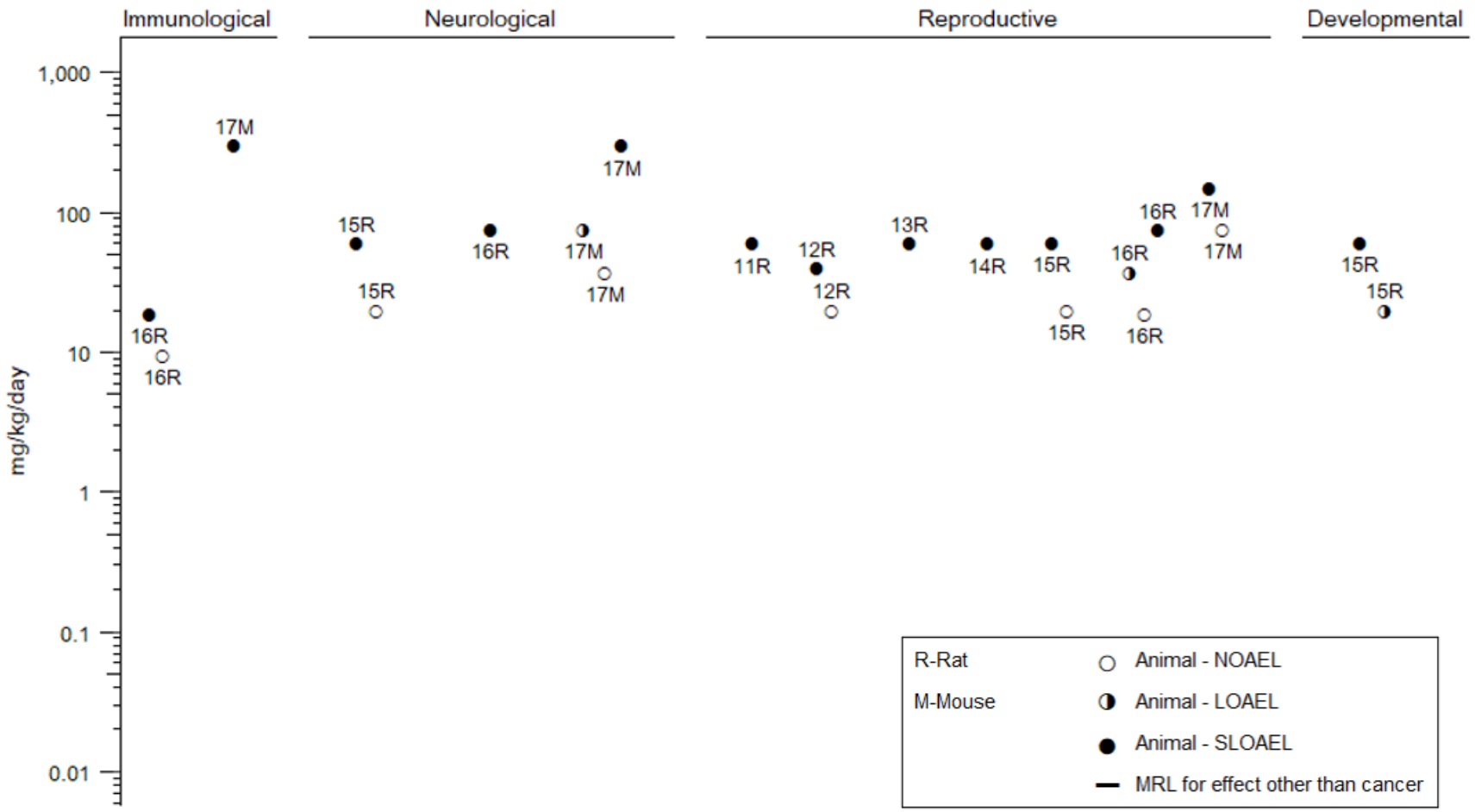

Table 2-3. Levels of Significant Exposure for Nitrobenzene – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE								
Rat (Fischer-344) 5 M, 5 F	12 days, daily	0, 0.2, 0.4, 0.8, 1.6, 3.2 g/kg	LE, CS, BW, HE, OW, GN, HP	Death			1.6	All animals died or were sacrificed moribund at ≥1.6 g/kg/day
				Bd wt	0.8			
				Resp	0.8	1.6		Lung congestion
				Hemato		0.2	0.4 M	LOAEL: Decreased hemoglobin, hematocrit, and erythrocyte counts (both sexes); increased WBCs (males); increased MCV and MCH (females); increased reticulocytes (both sexes); increased methemoglobin in males (7.672 versus 0.580% in controls) and females (6.924 versus. 0.644% in controls); minimal to mild spleen congestion SLOAEL: Cyanosis in males
				Hepatic		0.2		Increased liver weight
				Renal	0.8 F	1.6 F		Renal cortical tubule degeneration and cytoplasmic vacuolization in females
				Immuno			0.4	Lymphoid atrophy in males
				Repro	0.4 M		0.8 M	Seminiferous tubule atrophy; absence of spermatids and spermatozoa; multinucleated giant cells; decreased testes weight
NTP 1982								
Mouse (B6C3F1) 5 M, 5 F	12 days, daily	0, 0.2, 0.4, 0.8, 1.6, 3.2 g/kg	LE, CS, BW, HE, OW, GN, HP	Death			1.6	All animals died or were sacrificed moribund at ≥1.6 g/kg/day
				Bd wt	0.8			
				Hemato	0.2 M	0.2 F 0.4 M		Increased absolute and relative reticulocyte counts (both sexes); decreased hemoglobin concentration and erythrocyte counts (males)
				Hepatic	0.2 F 0.8 M	0.4 F		Increased liver weight
				Renal	0.8			
NTP 1982								

Table 2-3. Levels of Significant Exposure for Nitrobenzene – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
INTERMEDIATE EXPOSURE								
Rat (Fischer-344) 60 M, 60 F	90 days	0, 0.05, 0.1, 0.2, 0.4, 0.8 g/kg	BW, CS, GN, HP, LE, RX, FI, HE	Death			0.8	All animals died by week 11
				Bd wt	0.4			
				Resp	0.2 F 0.4 M	0.4 F		Lung congestion in females
				Cardio	0.2 M 0.4 F	0.4 M		Increased heart weight
				Hemato		0.05		Increased methemoglobin in males (0.985 versus 0.571% in controls) and females (0.995 versus 0.684% in controls); decreased erythrocyte count (females); spleen congestion
				Hepatic	0.1 F	0.1 M 0.2 F		Increased relative liver weight
				Renal	0.2	0.4		Kidney congestion
				Immuno			0.2	Lymphoid atrophy of spleen
				Neuro	0.05 F 0.4 M		0.1 F	Hemorrhages in the brain and brain stem; vacuolization in white matter, cerebellar white matter, and brain stem of females
				Repro	0.2 M		0.4 M	Markedly atrophic seminiferous tubules; hypospermatogenesis; multinucleate giant cells; >60% decrease in testes weight
NTP 1983b								
Mouse (B6C3F1) 60 M, 60 F	90 days	0, 0.05, 0.1, 0.2, 0.4, 0.8 g/kg	BW, CS, GN, HP, LE, RX, FI, HE	Death			0.8	9/10 males, 8/10 females died between weeks 3 and 10
				Bd wt	0.4			
				Hemato		0.05		Increased reticulocytes in males; increased methemoglobin in females (2.361 versus 1.357% in controls)
				Hepatic	0.2	0.4		Increased liver weight in females; cytomegaly in males
				Renal	0.1 M 0.4 F	0.2 M		Increased kidney weight in males

Table 2-3. Levels of Significant Exposure for Nitrobenzene – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
				Endocr	0.4 M	0.05 F		Fatty change in the adrenal cortex of females
				Immuno			0.8	Thymic atrophy
				Neuro			0.8	Brain stem hemorrhage and degeneration
				Repro	0.2 M	0.4 M 0.2 F		Decreased relative testes weight; testicular atrophy and hypospermatogenesis; uterine atrophy
NTP 1983b								

Bd wt or BW = body weight; Cardio = cardiovascular; CS = clinical signs; F = female(s); FI = food intake; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathological; Immuno = immunological; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; Neuro = neurological; NOAEL = no-observed-adverse-effect level; OW = organ weight; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; WBC = white blood cell

2.2 DEATH

No studies were located regarding lethal effects of nitrobenzene in humans after inhalation or dermal exposure. However, several case studies have reported deaths after nitrobenzene ingestion verified by blood analysis (Martínez et al. 2003) or where the substance was known to contain nitrobenzene (Gupta et al. 2000, 2012; Kumar et al. 2017). In the case reported by Martínez et al. (2003), an 82-year-old man ingested 250 mL of an unknown substance and developed severe (70%) methemoglobinemia; he died 4 days later. An analysis of blood collected 48 hours after ingestion showed that the nitrobenzene level was 3.2 µg/mL. Gupta et al. (2012) reported the case of a 17-year-old girl who was admitted to the hospital 6 hours after ingestion of an unknown quantity of nitrobenzene. The patient was unconscious, and her methemoglobin level was 63%. She was treated with oral methylene blue and intravenous vitamin C but died 4 days later (Gupta et al. 2012). In a case presented by Kumar et al. (2017), a 17-year-old girl who had accidentally ingested nitrobenzene 7 days prior to admittance to the hospital was treated with methylene blue for suspected acute methemoglobinemia. Sixteen days after she was hospitalized, she died of secondary aspiration pneumonitis, sepsis, and toxic brain injury due to nitrobenzene ingestion. Kumar et al. (2017) did not report the amount of nitrobenzene ingested or blood levels of nitrobenzene or methemoglobin. Gupta et al. (2000) briefly described the death of a 5-year-old boy who had ingested screen-printing material that contained nitrobenzene. Upon hospitalization, the boy was cyanotic, and was treated with gastric lavage and vitamin C. His condition deteriorated and the child expired about 26 hours after admission (Gupta et al. 2000). No information on the dose or blood levels of methemoglobin or nitrobenzene was reported.

Deaths have been reported in laboratory animals following acute-, intermediate-, and chronic-duration studies by several exposure routes. The available data suggest a steep dose-response curve for nitrobenzene lethality. For example, significant mortality was seen in rats and mice exposed by inhalation for 2 weeks to 124.5 ppm nitrobenzene, while there were no deaths at 35.8 ppm (Medinsky and Irons 1985). Similarly, in an intermediate-duration study of oral exposure, most male rats and male mice died prematurely at a dose of 150 mg/kg/day, while there were no deaths at 75 mg/kg/day (NTP 1983a). In acute- and intermediate-duration dermal exposure studies, the dose-response relationship for mortality was similar (NTP 1982, 1983b). All animals of both species died with dermal exposure to 1.6 g/kg but survived 0.8 g/kg in the acute-duration study (NTP 1982), and most animals of both species died with exposure to 0.8 g/kg but survived 0.4 g/kg in the intermediate-duration study (NTP 1983b).

In an unpublished acute lethality study, male Sprague-Dawley rats were exposed to 439, 514, 542, 555, 578, and 714 ppm of nitrobenzene 4 hours at a time (Dupont 1981). The following mortality incidences were reported: 0/10, 0/10, 1/10, 7/10, 8/10, and 10/10 rats for the low to high exposure concentrations, respectively. Most of the deaths occurred 1–2 days post exposure; one death occurred 7 days after exposure. The LC_{50} was calculated to be 556 ppm (Dupont 1981).

In a 14-day inhalation study comparing effects in Fischer 344 rats, CD rats, and B6C3F1 mice, species and strain differences in lethality were observed (Medinsky and Irons 1985). While all male and female Fischer-344 rats survived 2 weeks of exposure to 124.5 ppm, five male and three female CD rats and all B6C3F1 mice of both sexes exposed to this concentration died or were humanely sacrificed by the 4th day of exposure. The deaths of CD rats and B6C3F1 mice were attributed to perivascular hemorrhage in the cerebellar peduncle (Medinsky and Irons 1985). Pregnant CD rats survived acute-duration exposure on gestation days (GDs) 6–15 (6 hours/day) to concentrations up to 39.4 ppm (Tyl et al. 1987). Similarly, no treatment-related mortalities were observed when pregnant New Zealand white rabbits were exposed to nitrobenzene concentrations up to 104 ppm for 6 hours/day during GDs 7–19 (Biodynamics 1983, 1984).

In intermediate-duration inhalation studies, F344 and CD rats and B6C3F1 mice of both sexes survived exposure to nitrobenzene concentrations up to 48.7 ppm for 90 days (6 hours/day, 5 days/week). In addition, 2 years of inhalation exposure to concentrations up to 24.8 ppm (F344 and CD rats) or 49.1 ppm (B6C3F1 mice) did not alter survival rates of either species (Cattley et al. 1994, 1995; CIIT 1993).

The oral LD_{50} of nitrobenzene in female albino rats exposed by gavage was estimated to be 600 mg/kg (Smyth et al. (1969). Only one other acute-duration oral study reported animal deaths; in a study reporting multiple experiments, the study authors reported that 8.5% of female B6C3F1 mice (across several experiments) given 300 mg/kg/day nitrobenzene in corn oil for 14 days died (Burns et al. 1994). No deaths were reported among male Sprague-Dawley rats exposed for 14 days to oral doses of 60 mg/kg/day (Kawashima et al. 1995).

In intermediate-duration oral studies, significant mortality was reported at doses ≥100 mg/kg/day in rats or 300 mg/kg/day in mice. Mitsumori et al. (1994) reported that 2/10 males and 9/10 female Sprague-Dawley rats exposed to 100 mg/kg/day by gavage died before scheduled termination in a combined repeat-dose and reproductive/developmental screening study. The causes of death were not reported. The study authors also reported the deaths of 1/10 females in each of the 20 and 60 mg/kg/day dose groups during the lactation period. It is not clear whether the deaths at the latter doses were related to treatment,

but females in this group were noted to exhibit anemia (6/10) and neurological signs of toxicity (ataxia and torticollis, 1/10) (Mitsumori et al. (1994). In a 90-day gavage study, 9/10 male and 3/10 female F344 rats given doses of 150 mg/kg/day died prior to study completion (NTP 1983a). In the same study, exposure to doses of 300 mg/kg/day resulted in premature death of 3/10 male mice and moribund sacrifice of another 2/10 male mice (NTP 1983a).

In an acute-duration dermal study, Fischer 344 rats and B6C3F1 mice were exposed to nitrobenzene via daily dermal application for 12 days. All rats and mice of both sexes exposed to doses of 1.6 and 3.2 g/kg of nitrobenzene either died or were sacrificed moribund (NTP 1982). When Fischer 344 rats and B6C3F1 mice were exposed by dermal application in a 13-week intermediate-duration study, all rats, 9/10 male mice, and 8/10 female mice exposed to 0.80 g/kg died by week 11 (NTP 1983b).

2.3 BODY WEIGHT

No human studies were located evaluating body weight effects of nitrobenzene exposure following inhalation, oral, or dermal exposure. Few animal studies have reported changes in body weight associated with nitrobenzene exposure. Male Sprague-Dawley rats exposed to 439–714 ppm of nitrobenzene for a single 4-hour period in an acute lethality study experienced weight losses of 8–21% of initial body weight 1–4 days after exposure (Dupont 1981). In gestational exposure studies, pregnant rats and rabbits exhibited reduced weight gain or weight loss. Decreased maternal weight gain was observed in CD rats exposed to 39.4 ppm for 6 hours/day on GDs 6–15 (Tyl et al. 1987). In pregnant rabbits exposed to 40 ppm nitrobenzene for 6 hours/day during GDs 7–19, there was a slight mean weight loss during gestation; the change was not statistically significant relative to controls (Biodynamics 1984). Body weights were not measured in the 14-day inhalation experiments in F344 rats, CD rats, and B6C3F1 mice conducted by Medinsky and Irons (1985).

No effects on body weights were reported in B6C3F1 mice or F344 or CD rats exposed to concentrations of up to 48.7 ppm for 90 days (Hamm et al. 1984). In a 2-year inhalation study of B6C3F1 mice and Fischer 344 and CD rats, no effects on body weight were observed at concentrations up to 49.1 ppm in mice or 24.8 ppm in rats (Cattley et al. 1994, 1995; CIIT 1993). Statistically significant fluctuations in mean body weights occurred over the course of the 2-year study but they were not exposure-related, and differences from control body weights did not reach 10%.

Female B6C3F1 mice administered 300 mg/kg/day nitrobenzene for 14 days via gavage displayed a 12% increase in body weight (compared with controls), which the study authors attributed to fluid retention (Burns et al. 1994). Female mice receiving 100 mg/kg/day in the same study did not have any body weight differences from controls (Burns et al. 1994). Male Sprague-Dawley rats given 100 mg/kg/day nitrobenzene orally in a combined repeat-dose and reproductive/developmental screening study exhibited a 17% decrease in body weight at termination (Mitsumori et al. 1994). For females in this study, body weight changes were reported qualitatively. The study authors reported that females given 100 mg/kg/day consumed less food prior to mating and during pregnancy, with a consequent decrease in body weight gain on day 21 of pregnancy. Maternal body weight gain was also decreased during the lactation period at this dose. Females given 60 mg/kg/day also showed an inhibition of body weight gain during the lactation period, accompanied by a decrease in food consumption (Mitsumori et al. 1994). In 90-day studies of Fischer 344 rats and B6C3F1 mice exposed orally, mean final body weight was not significantly different in rats exposed to doses up to 75 mg/kg/day or mice exposed to doses up to 300 mg/kg/day when compared to controls. The only surviving male rat in the 150 mg/kg/day group did have a significant decrease in body weight (>10% compared to control) (NTP 1983a).

In studies using dermal application of nitrobenzene, Fischer 344 rats and B6C3F1 mice exposed to doses up to 0.8 g/kg for 12 days or up to 0.4 g/kg for 90 days showed no significant changes in body weight (NTP 1982, 1983b).

2.4 RESPIRATORY

No studies were located in humans evaluating the respiratory effects of nitrobenzene through inhalation, oral, or dermal exposure routes.

In an acute-duration, 2-week inhalation study in male and female F344 and CD rats and B6C3F1 mice (Medinsky and Irons 1985), mice exhibited moderate bronchiolar epithelial hyperplasia after exposure to 124.5 ppm nitrobenzene (a concentration that resulted in death or humane sacrifice within the first 4 days of exposure) and mild bronchiolar hyperplasia after exposure to 35.8 ppm (all mice survived to termination). Male and female CD rats exposed to 124.5 ppm nitrobenzene also died or were sacrificed moribund within the first exposure week; in these animals, perivascular edema and vascular congestion in the lungs were found (Medinsky and Irons 1985). In a developmental toxicity study, Biodynamics (1984) reported a high incidence of discolored lungs in New Zealand White rabbits in all exposure groups (concentrations from 10 to 81 ppm were administered on GDs 7–19).

Hamm et al. (1984) observed treatment-related lesions in the lungs of F344 and CD rats and B6C3F1 mice exposed to nitrobenzene by inhalation for 90 days. There was a minimal to slight hyperplasia in the bronchial epithelium in male F344 rats and in both male and female mice after exposure to 48.7 ppm. Female rats did not show this effect of treatment. In male CD rats, rhinitis associated with epithelial and goblet cell hyperplasia was observed in nasal turbinates after exposure to 48.7 ppm nitrobenzene (Hamm et al. 1984).

In a 2-year study of the toxicity of inhaled nitrobenzene, Cattley et al. (1994, 1995; CIIT 1993) observed lesions in the nasal turbinates and lungs of mice and in the nasal turbinates of male CD rats. In mice, a marked increase in bronchiolization of alveolar walls at all exposure concentrations (≥5 ppm), and males showed increased alveolar/bronchiolar hyperplasia at ≥24.8 ppm (Cattley et al. 1994, 1995; CIIT 1993).

Nasal lesions occurred at all exposure concentrations (≥1 ppm) in rats including olfactory epithelial pigment deposition (F344 and CD rats) and squamous epithelial hyperplasia (CD rats). At higher exposures, nasal findings in the rats of both strains included inflammation, sometimes with submucosal gland hypertrophy, and suppurative exudate. Mice exhibited more severe lesions in the nasal passages, including the following findings that were seen at significantly increased incidences at all exposure levels (≥5 ppm): glandularization of the respiratory epithelium, olfactory epithelial degeneration, increased secretory product in the respiratory epithelium, olfactory epithelial pigment deposition, and dilatation of the submucosal glands.

A significant increase in absolute lung weight was reported when B6C3F1 mice were gavaged with ≥30 mg/kg/day nitrobenzene for 14 days; however, relative lung weight was increased only at the high dose (300 mg/kg/day) (Burns et al. 1994). Increased lung weights were also reported in female F344 rats after 90 days of oral exposure to ≥75 mg/kg/day (NTP 1983a).

Dermal exposure to nitrobenzene for 12 or 90 days resulted in lung congestion in F344 rats, but not B6C3F1 mice (NTP 1982, 1983b). In rats, 12-day exposure to 1.6 g/kg induced this effect in both sexes, while a significant increase was seen only in females exposed for 90 days to 0.4 g/kg (NTP 1982, 1983b).

2.5 CARDIOVASCULAR

No relevant studies were located regarding cardiovascular effects in humans or animals after inhalation exposure to nitrobenzene. In a case study of a 32-year-old male exposed orally to nitrobenzene, cardiogenic pulmonary edema was noted (Agrawal et al. 2011). The amount of nitrobenzene ingested was not known.

Increased incidences of mineralization of the aorta and myocardium were noted in male CD rats exposed for 2 years to 24.8 ppm nitrobenzene; however, these lesions were considered secondary effects of severe chronic nephropathy at this exposure level (Cattley et al. 1994, 1995; CIIT 1993). No cardiovascular effects were noted in rats or mice exposed to nitrobenzene by inhalation in other acute-, intermediate-, or chronic-duration studies (Medinsky and Irons 1985; Hamm et al. 1984; Cattley et al. 1994, 1995; CIIT 1993). No acute-duration oral studies included assessment of cardiovascular tissues. Of the available oral studies, only the 90-day studies by NTP (1983a) reported results of endpoints relevant to cardiovascular health. Increased absolute and relative heart weights were observed in F344 female rats at all dose levels (≥9.375 mg/kg/day) after 90 days of exposure via gavage (NTP 1983a). There were no histopathology correlates in the hearts of female rats, and no treatment-related changes in heart weights or histopathology were observed in male rats or in male or female mice (NTP 1983a).

In the 90-day dermal exposure study of rats and mice, increased absolute and relative heart weights were noted in male rats exposed to 0.4 g/kg (the highest dose that animals survived), but not in female rats or in male or female mice at the same dose (NTP 1983b). No treatment-related lesions were observed in the hearts of rats or mice in this study.

2.6 GASTROINTESTINAL

No studies were located regarding gastrointestinal effects in humans after inhalation, oral, or dermal exposure to nitrobenzene. Female F344 rats exposed to concentrations ≥5 ppm nitrobenzene for 90 days by inhalation exhibited diarrhea, but male rats and mice did not exhibit this effect (Hamm et al. 1984). Chronic inhalation exposure to 24.8 ppm nitrobenzene resulted in an increased incidence of focal pancreatic acinar cell hyperplasia in male F344 rats (Cattley et al. 1994, 1995; CIIT 1993). No other reports of gastrointestinal effects in animals exposed to nitrobenzene were located.

2.7 HEMATOLOGICAL

Overview. A key event in the mechanism by which nitrobenzene induces many of its toxic effects is via conversion of the iron component of hemoglobin from the ferrous state to the ferric state (oxidized), forming methemoglobin. Methemoglobin is not capable of binding and transporting oxygen to the tissues of the body, leading to tissue hypoxia. Methemoglobin occurs naturally in people, at levels around 1–4% in blood (Smith and McHale 2018). However, increases in the amount of methemoglobin cause cyanosis (slate blue coloration), fatigue, weakness, dyspnea, headache, and dizziness. The severity of effects of methemoglobinemia in humans increase with the percentage in the blood (Ludlow et al. 2021):

- At ≥10%, cyanosis may be evident in a healthy person.
- At ≥15%, the characteristic "chocolate brown blood" may be present.
- As the level approaches 20%, symptoms of anxiety, light-headedness, and headaches may occur.
- At levels in the range of 30–50%, there may be tachypnea, confusion, and loss of consciousness.
- As the level approaches 50%, there is risk of seizures, dysrhythmias, metabolic acidosis, and coma.
- Levels above 70% are often fatal in humans.

The mechanism by which nitrobenzene induces methemoglobinemia is well-studied and is a result of redox cycling of its metabolites (see Metabolic Mechanisms in Section 3.1.3). The action of bacteria normally present in the small intestine and gut is an important element in the formation of methemoglobin resulting from nitrobenzene exposure (Goldstein et al. 1984; Reddy et al. 1976). Germ-free rats do not develop methemoglobinemia when exposed to nitrobenzene by oral administration (Reddy et al. 1976).

Many of the effects induced by nitrobenzene result from production of methemoglobin, along with the destruction of erythrocytes and oxidative stress. Hematology changes include decreases in red blood cell counts and decreases in hemoglobin concentration. These effects trigger stimulation of hematopoiesis in the bone marrow, increases in reticulocyte counts, and extramedullary hematopoiesis in the spleen and liver. As the spleen is the primary site where damaged erythrocytes are scavenged, splenic congestion and hemosiderosis are common findings, and may result in relative depletion of lymphoid cells. The liver may also participate in phagocytosis of damaged red blood cells, and this organ is where heme is recycled, leading to increases in bilirubin as the heme molecule is broken down. Some renal effects of nitrobenzene may also be attributable to its hematotoxicity, as free hemoglobin from lysed erythrocytes

can form nephrotoxic dimers. In addition, due to their roles in elimination of damaged red blood cells, the spleen, liver, and kidneys are also exposed to oxidants released from these cells. As detailed below and in Tables 2-1, 2-2, and 2-3, studies in laboratory animals exposed by inhalation, oral, and dermal routes have demonstrated all of these effects.

Human Studies. Case studies reported elevated methemoglobin levels in workers exposed to nitrobenzene via inhalation in the workplace (Ikeda and Kita 1964; Lee et al. 2013). Airborne concentrations of nitrobenzene to which the workers were exposed were not reported. In addition, numerous cases of methemoglobinemia in humans have been reported due to oral exposure to nitrobenzene (Agrawal et al. 2011; Balwani et al. 2017; Boukobza et al. 2015; Chongtham et al. 1997; D'sa et al. 2014; Kumar et al. 1990; Perera et al. 2009; Saxena and Prakash Saxena 2010), including several that resulted in fatalities (Gupta et al. 2000, 2012; Kumar et al. 2017; Martínez et al. 2003). Mallouh and Sarette (1993) reported the case of a 2-month-old infant who was exposed by dermal contact with hair oil containing 1% nitrobenzene. The infant had a methemoglobin level of 31.5%, which gradually dropped over 3 days without treatment (Mallouh and Sarette). Additionally, Ewert et al. (1998) described methemoglobinemia and related symptoms occurring in a 33-year-old man who attempted suicide by intravenously injecting "India ink," which contained nitrobenzene. Blood and urine samples confirmed the presence of nitrobenzene.

Animal Studies. As with humans, dose-related increases in the amount of methemoglobin in the blood have been shown in studies of rats, mice, and rabbits after acute-, intermediate-, and/or chronic-duration exposures to nitrobenzene (Biodynamics 1983, 1984; Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984; Medinsky and Irons 1985; Mitsumori et al. 1994; NTP 1982, 1983a, 1983b).

In acute-duration, 2-week inhalation exposure studies in rats and mice (Medinsky and Irons 1985), hematology, spleen weight, and splenic histopathology changes attributable to nitrobenzene exposure were observed at all exposure levels (9.1, 35.8, and 124.5 ppm). In this study, half of each group (5/species/sex/exposure level) were sacrificed 3 days after the end of exposure and the other half were sacrificed 14 days after exposure termination. Blood samples were collected for hematology and methemoglobin analysis at sacrifice. Methemoglobin levels were statistically significantly increased only in female CD rats exposed to 124.5 ppm from the group scheduled to be sacrificed three days after the end of exposure (Medinsky and Irons 1985). However, all CD rats exposed to 124.5 ppm were reported to have died or been humanely sacrificed at the end of the first week of exposure; thus, the timing of the blood sample collection and methemoglobin analysis is uncertain. The lack of effect on methemoglobin

in surviving animals of other exposure groups could have resulted from recovery occurring during the post-exposure period, or from a delay in analysis after sampling.

Hematologic effects seen in the exposed animals included decreased red blood counts in F344 and CD rats at all exposure levels; other hematology changes were restricted to the highest exposure group (124.5 ppm) in which all CD rats and all mice died or were euthanized early (Medinsky and Irons 1985). Concentration-dependent increases in relative spleen weight were observed in rats, with marked increases at higher exposure concentrations; organ weights were not reported for mice. At the lowest exposure level (9.1 ppm), spleen weights measured 3 days after the end of exposure were increased by 33% in female F344 rats and 44% in female CD rats. Male rats of both strains exhibited significant increases at ≥35.8 ppm of at least 74% relative to controls. The study authors stated that "splenic lesions were evident in all animals and dose groups exposed to nitrobenzene;" however, Medinsky and Irons (1985) reported quantitative histopathology data only for the 35.8 and 124.5 ppm groups and not for the control or 9.1 ppm exposure groups. As a result, the specific nature and incidences of splenic lesions in the 9.1 ppm groups are not known. At 35.8 ppm, nearly all animals of all sexes and strains (9/10 or 10/10 per group) showed extramedullary hematopoiesis in the spleen. In addition, all rats of both strains and sexes had hemosiderosis, while all F344 rats and about half of the CD rats, along with half of the female mice showed sinusoidal congestion. At 124.5 ppm, 90–100% of rats and mice examined demonstrated hemosiderin-laden macrophages in red pulp, extramedullary hematopoiesis, and acute congestion of the spleen (Medinsky and Irons 1985).

Many of the same effects were also seen with 90 days of inhalation exposure to nitrobenzene in mice (B6C31) and rats (Sprague-Dawley and Fischer 344) (Hamm et al. 1984). Increased serum methemoglobin was observed at all exposure levels (≥5 ppm) in male F344 rats; at ≥15.8 ppm in female F344 and male CD rats; and at 48.7 ppm in female CD rats and mice of both sexes. Hematology changes indicative of hemolytic anemia were seen in rats but not mice. Decreased erythrocyte counts, hematocrit, and/or hemoglobin, and increased erythrocyte width were evident at all exposure concentrations (≥5 ppm) in female F344 rats, at ≥15.8 ppm in male and female CD rats, and at 48.7 ppm in male F344 rats. Male and female F344 rats exhibited increased absolute spleen weights at ≥15.8 ppm; similarly, male and female CD rats had increased absolute and relative spleen weights (≥15.8 ppm in females and at 48.7 ppm in males). Spleen weights were increased in male and female mice exposed to 48.7 ppm (Hamm et al. 1984). Increased incidences of splenic lesions were observed at all levels in both rats and mice of both sexes. Apart from male mice, all animals in all treatment groups exhibited sinusoidal congestion in the spleen, while this finding was absent in all control groups. Male and female F344 rats and male CD rats

also exhibited increased severity of hemosiderin deposition at all exposure concentrations (with severity score generally increasing from slight in controls to moderate at 5 ppm). In F344 rats, additional treatment-related effects seen at the highest concentration (48.7 ppm) included proliferation of mesenchymal cells in the spleen, fibroblastic hyperplasia of the splenic capsule, accumulation of lymphocytes and macrophages in the spleen, extramedullary hematopoiesis in the spleen, and bone marrow hyperplasia. Male and female mice of all treatment groups showed increased incidences (but not severity) of hemosiderin deposition in the spleen. At the highest exposure level in B6C3F1 mice, increased bone marrow hyperplasia was observed (Hamm et al. 1984).

The hematology and histopathology changes observed in the acute- and intermediate- duration inhalation studies were also observed in a chronic-duration, 2-year study of the same species. In this study, F344 (both sexes) and CD (male) rats were exposed to 0, 1, 5, and 24.8 ppm of nitrobenzene and B6C3F1mice (both sexes) to 0, 5, 24.8, and 49.1 ppm nitrobenzene (Cattley et al. 1994, 1995; CIIT 1993). Male and female F344 rats exhibited decreased erythrocyte counts, hematocrit, and hemoglobin as well as increased methemoglobin at 24.8 ppm; these changes were evident at both the interim and final sacrifices in F344 rats. At the interim sacrifice, male CD rats exhibited increased methemoglobin at all exposure levels (≥1 ppm), while at termination the difference from control was significant only at 24.8 ppm. Spleen weights were not altered by exposure at any concentration in rats. Increased incidences of spleen congestion were reported in male and female F344 rats and male CD rats at all exposure levels (≥1 ppm), and increased spleen pigmentation was reported at all exposure levels in male F344 rats. In male mice, the hematology changes consisted of decreased erythrocyte counts and hematocrit, and increased methemoglobin at 49.1 ppm. In female mice, methemoglobin was increased at ≥24.8 ppm (Cattley et al. 1994, 1995; CIIT 1993). No changes in spleen weights were observed in mice. In addition, splenic lesions were not observed in male mice, but females showed an increased incidence of lymphoid hyperplasia in the spleen at 49.1 ppm. Finally, an increased incidence of bone marrow hypercellularity was reported for male mice exposed to 49.1 ppm (Cattley et al. 1994, 1995; CIIT 1993).

In a 14-day immunotoxicity study of oral exposure to nitrobenzene in female B6C3F1 mice, exposure to nitrobenzene at all doses (≥30 mg/kg/day) resulted in proliferative effects on the bone marrow, measured as increased deoxyribonucleic acid (DNA) synthesis and increased numbers of cells per femur (Burns et al. 1994). The numbers of granulocyte-monocyte colony-forming units (CFUs) were increased when expressed as number per femur, but not when expressed as number per 10^5 bone marrow cells. The study authors noted that the assays did not distinguish the specific cell population(s) responsible for the increase in DNA synthesis (Burns et al. 1994). However, a dose-dependent increase in the numbers of peripheral

blood reticulocytes was also observed in exposed mice, with increases of >3-fold (relative to controls) in the 100 and 300 mg/kg/day groups, suggesting stimulation of bone marrow erythropoiesis. The immature erythrocytes (reticulocyte) resulted in compensatory increases in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) starting at doses of 100 mg/kg. Hepatomegaly and splenomegaly were observed by the researchers, as were pathological observations consistent with extramedullary hematopoiesis.

In a repeat-dose and reproductive/developmental toxicity screening study, Mitsumori et al. (1994) observed significant hematological effects in male Sprague-Dawley rats with 54 days of oral exposure to nitrobenzene at doses ranging from 20 to 100 mg/kg/day. Dose-dependent increases in methemoglobin and decreases in erythrocyte count, hemoglobin concentration, and hematocrit were seen at all doses (≥20 mg/kg/day). At doses ≥60 mg/kg/day, there were significant increases in MCV, mean erythrocyte hemoglobin concentration, reticulocytes, and erythroblasts. Absolute and relative spleen weights were increased at all dose levels (≥60% relative to controls). Mitsumori et al. (1994) also observed extramedullary hematopoiesis and hemosiderin deposition in the spleen and increased hematopoiesis in bone marrow in all treated rats at all dose levels (≥20 mg/kg/day).

In intermediate-duration oral toxicity studies in rats and mice conducted by NTP (1983a), B6C3F1 mice and F344 rats of both sexes were exposed to doses ranging from 9.375 to 150 mg/kg/day for rats and from 18.75 to 300 mg/kg/day for mice. Because only one male rat survived in the 150 mg/kg/day dose group, results from this group are not informative. Mice and rats of both sexes had increased methemoglobin levels at all doses. Rats of both sexes had increased reticulocytes and decreased hemoglobin at ≥9.375 mg/kg/day. In addition, male rats had decreased MCV and MCH, while female rats had decreased hematocrit at all doses. Increased reticulocytes were observed in female mice at ≥18.75 mg/kg/day and in males at ≥37.5 mg/kg/day; decreases in red blood cells, hematocrit, and hemoglobin were seen in both sexes at ≥150 mg/kg/day. Male mice exhibited anisocytosis and polychromasia at 300 mg/kg/day. Histological effects included congestion of the spleen in most of the treated rats of both sexes. Hemosiderin pigment was observed in the red pulp of the spleen, while lymphoid depletion was noted in the white pulp. In mice, lymphoid depletion of the spleen was noted at ≥150 mg/kg/day.

Dermal exposure studies have demonstrated effects very similar to those seen in inhalation and oral studies. NTP (1982) noted increased methemoglobin; decreased hemoglobin, hematocrit, and erythrocyte counts; increased MCV, MCH, reticulocytes; and spleen congestion at doses of 0.2 g/kg when nitrobenzene was applied to the skin of the intrascapular region of F344 rats for 12 days. B6C3F1 mice

exhibited decreased hemoglobin concentrations and erythrocyte counts, along with increased reticulocytes at ≥0.2 g/kg (NTP 1982). In a 90-day dermal exposure study with both F344 rats and B6C3F1 mice, increased methemoglobin was seen in both species and sexes, and changes consistent with hemolytic anemia (decreased hemoglobin and erythrocyte counts, increased reticulocytes) were observed at all doses (≥0.05 g/kg) (NTP 1983b). Spleen congestion was seen in all groups of treated rats but only in mice exposed to the highest dose (0.8 g/kg).

2.8 MUSCULOSKELETAL

No studies were located regarding musculoskeletal effects in humans or animals after inhalation, oral, or dermal exposure to nitrobenzene. In the chronic-duration inhalation study of nitrobenzene (Cattley et al. 1994, 1995; CIIT 1993), increased incidences of mineralization of the stomach muscle and fibrous osteodystrophy of the nose and bone were observed in male CD rats at the highest exposure concentration (24.8 ppm). These changes were considered secondary effects of the severe chronic nephropathy observed at this exposure level (CIIT 1993).

2.9 HEPATIC

There is some evidence that the human liver is affected after exposure to nitrobenzene. Ikeda and Kita (1964) reported that a woman who was occupationally exposed via inhalation to nitrobenzene for 17 months had an enlarged and tender liver; liver function tests showed marked retention of BSP and slight increases in icterus index and indirect bilirubin level (Ikeda and Kita 1964). Nitrobenzene exposure levels were not measured or estimated. Gupta et al. (2012) presented a case of a 17-year-old female who died by suicide after consuming an unknown quantity of nitrobenzene, resulting in severe methemoglobinemia. At autopsy, hepatic centrilobular necrosis was observed in the patient (Gupta et al. 2012). No studies were located regarding hepatic effects in humans after dermal exposure to nitrobenzene.

Treatment-related liver effects, including increased liver weights, clinical chemistry changes, and a wide range of histopathology changes (including necrosis, degeneration, eosinophilic foci, basophilia, spongiosis hepatis, and hepatocellular hypertrophy) have been observed in rats and mice exposed by inhalation, oral, and dermal routes. In addition to these changes, some studies have reported hepatic effects such as Kupffer cell pigmentation that likely stem from nitrobenzene-induced erythrocyte

destruction. Studies in rats and mice show species, strain, and sex differences in the nature and severity of liver effects induced by nitrobenzene.

In acute-duration studies of F344 rats, CD rats, and B6C3F1 mice exposed by inhalation for 14 days, Medinsky and Irons (1985) observed increased liver weights in male F344 rats exposed to nitrobenzene concentrations ≥9.1 ppm and in female F344 rats exposed to concentrations ≥35.8 ppm. Upon microscopic examination, however, there were no histopathology changes in the livers of F344 rats. In contrast, no liver weight changes were seen in CD rats of either sex at the same exposure levels, but male CD rats exhibited mild hepatocyte necrosis at 35.8 ppm. At the highest exposure level (124.5 ppm), all male and female CD rats died or were sacrificed moribund by the end of the first week; these rats exhibited sinusoidal congestion, along with centrilobular hydropic degeneration and periportal basophilic hepatocyte degeneration (Medinsky and Irons 1985).

In developmental toxicity studies, maternal liver weight did not differ significantly from controls when pregnant CD rats were exposed to concentrations up to 39.4 ppm on GDs 6–15 (Tyl et al. 1987) or when pregnant New Zealand white rabbits were exposed to concentrations up to 104 ppm on GDs 7–19 (Biodynamics 1983, 1984). No other maternal hepatic endpoints were evaluated in the developmental toxicity studies.

Hamm et al. (1984) conducted a 90-day inhalation exposure study in B6C3F1 mice and CD and F344 rats exposed to nitrobenzene concentrations of 5, 15.8, and 48.7 ppm. Increased absolute and relative liver weights were observed at ≥15.8 ppm in male and female F344 rats. Histopathology changes in F344 rats were restricted to the highest exposure group; both males and females exposed to 48.7 ppm exhibited disorganized hepatic cords, vascular ectasia in the liver, and centrilobular hepatocyte degeneration. Male F344 rats also had increased incidences of periportal hepatocyte basophilia while females had increased incidences of focal necrosis. CD rats were somewhat more sensitive to the hepatic effects of nitrobenzene. In male CD rats, increased incidences of microgranulomas in the liver were noted at all exposure concentrations (≥5 ppm), without changes in liver weight. Female CD rats showed increased absolute and relative liver weights as well as increased incidences of centrilobular hepatocyte hypertrophy ≥15.8 ppm (Hamm et al. 1984). Intermediate-duration inhalation exposure to nitrobenzene at concentrations up to 15.8 ppm did not result in liver effects in mice (Hamm et al. 1984). At the highest concentration (48.7 ppm), liver weights were increased in both male and female mice; serum alanine aminotransferase (ALT) was increased by ~2-fold in male mice; and increased incidences of hepatic

lesions were seen (centrilobular hepatocyte hyperplasia with some cord disorganization in females and basophilic hepatocytes in males).

In the 2-year study of inhalation exposure, the liver was a target organ for nitrobenzene in both rats and mice (Cattley et al. 1994, 1995; CIIT 1993). In male F344 rats, increased incidences of eosinophilic foci and centrilobular hepatocytomegaly were observed at concentrations ≥5 ppm, while increases in liver weight and an increased incidence of spongiosis hepatis were seen at 24.8 ppm. Increased liver weights and increased incidences of eosinophilic foci and spongiosis hepatis were noted in female F344 rats exposed to 24.8 ppm; no hepatic effects were seen at lower exposure levels in the females. Male CD rats showed increased incidences of pigmentation in the Kupffer cells at all exposure levels (≥1 ppm) (Cattley et al. 1994, 1995; CIIT 1993). In addition, increased centrilobular hepatocytomegaly and increased spongiosis hepatis occurred in male CD rats at ≥5 and 24.8 ppm, respectively. In mice, increased incidences of centrilobular hepatocytomegaly and multinucleated hepatocytes were reported in males at all exposure levels (≥5); females exhibited an increased incidence of centrilobular hepatocytomegaly only at the highest exposure (49.1 ppm) (Cattley et al. 1994, 1995; CIIT 1993). In rats and female mice, nitrobenzene exposure also resulted in dose-related trends or significant increases in the incidences of liver tumors, while liver tumor incidences were not altered by exposure in male mice (see Section 2.19, Cancer).

Female B6C3F1 mice exposed to nitrobenzene by gavage for 14 days in a series of immunotoxicity experiments showed increased liver weight and hepatomegaly at doses ≥100 mg/kg/day (Burns et al. 1994). Further, at 300 mg/kg/day (a dose at which 8.5% of mice died), serum ALT was significantly increased by >2-fold, and minor histopathological changes including mild hydropic degeneration around the focal central veins were seen in the female mice (Burns et al. 1994). In a repeated-dose and reproductive/developmental toxicity screening study (Mitsumori et al. 1994), male Sprague-Dawley rats exposed for 54 days to doses ≥20 mg/kg/day exhibited increased liver weights and centrilobular hepatocyte swelling, along with increased incidences of microscopic lesions indicative of hemolysis (hemosiderin deposition in Kupffer cells and extramedullary hematopoiesis). Liver weights and histopathology were not evaluated in females in this study (Mitsumori et al. 1994).

With longer (90-day) oral exposure, hepatic effects were seen at lower doses. NTP (1983a) reported increases in liver weights at all doses in F344 rats (≥9.375 mg/kg/day); however, no treatment-related increases in the incidences of histopathology findings were observed at these doses. At the highest dose (150 mg/kg/day), which resulted in premature mortalities in 9/10 males and 3/10 females, congestion in

the liver was seen (NTP 1983a). Increases in liver weight without histopathology changes were observed in female mice at all doses (≥18.75 mg/kg/day) in the 90-day gavage study (NTP 1983a). In male mice, liver weight increased at 150 and 300 mg/kg/day, and there was a significant increase in the incidence of hepatocellular cytomegaly at 300 mg/kg/day. Mortalities among male mice at 300 mg/kg/day limits interpretation of hepatic effects at that dose (NTP 1983a). Cytomegaly was observed in one and two male mice each in the 75 and 150 mg/kg/day groups, respectively (NTP 1983a).

In acute-duration (12 days) and intermediate-duration (90 days) studies of dermal exposure to nitrobenzene, similar hepatic effects were reported. Both F344 rats and female B6C3F1 mice exhibited increased liver weights after 12 days of dermal exposure (NTP 1982). Rats of both sexes showed higher liver weights at doses of ≥0.2 g/kg. The only dose-related histopathology finding in the liver of rats was an increase in the incidence of mild hematopoiesis in males. In female mice, increased liver weights occurred at doses ≥0.4 g/kg, while male mice showed a significant increase in relative (to body weight) liver weight at 0.8 g/kg (NTP 1982). Histopathology examination in the mice was limited to the 1.6 g/kg group, and all mice died or were sacrificed moribund prior to study termination in this group. Hepatic congestion was reported in a few of the decedents at this dose (NTP 1982).

After 90 days of dermal exposure to nitrobenzene, male and female F344 rats exhibited increased relative liver at ≥0.1 and ≥0.2 g/kg, respectively (NTP 1983b). Histopathology findings in the liver were confined to the high dose of 0.8 g/kg, a dose at which all rats of both sexes died prematurely. Findings in the livers of the decedents included hepatic congestion with blood pooled in the hepatic artery, portal and collecting veins, and sinusoids (NTP 1983b). In mice exposed for 90 days by dermal application of 0.4 g/kg, increased absolute and relative liver weights were seen in females and increased absolute (but not relative) liver weight was observed in males (NTP 1983b). No microscopic lesions were observed in the livers of female mice at 0.4 g/kg, but all male mice exposed to this dose exhibited hepatic cytomegaly. At the high dose of 0.8 g/kg, which also resulted in significant mortality in mice, 8/10 female mice showed hepatocellular cytomegaly, and 6/10 male mice had yellow pigmented cells in the liver (NTP 1983b).

2.10 RENAL

No studies were located regarding renal effects in humans after inhalation or dermal exposure to nitrobenzene. One publication (Gupta et al. 2012) reported a case of renal tubular necrosis identified at autopsy following the death of a 17-year-old girl by nitrobenzene ingestion. The amount of nitrobenzene ingested was unknown.

In developmental toxicity studies of rats and rabbits exposed by inhalation during gestation, no effects on maternal kidney weights were observed at concentrations up to 39.4 and 104 ppm, respectively (Tyl et al. 1987; Biodynamics 1983, 1984). Renal histopathology was not evaluated in these studies.

Dose-related increases in kidney weights were observed in F344 rats of both sexes exposed to nitrobenzene via inhalation for 14 days (Medinsky and Irons 1985). The increases were statistically significant at concentrations ≥9.1 ppm in males and at ≥35.8 ppm in females in animals sacrificed 3 days after the end of exposure; the changes were no longer evident in the groups sacrificed 14 days after the end of exposure (Medinsky and Irons 1985). All (10/10) male and two (2/10) female F344 rats exposed to 124.5 ppm nitrobenzene exhibited moderate to severe hyaline nephrosis that also regressed after the recovery period. Kidney weight changes were not observed at any exposure concentration in male or female CD rats in this study (Medinsky and Irons 1985). At 124.5 ppm, all CD rats died or were sacrificed moribund by the end of the first week of exposure; in these animals, moderate to severe hydropic degeneration of the cortical tubular cells was a frequent finding. Male CD rats that died or were humanely sacrificed after exposure to this concentration also exhibited degenerative changes in the kidneys (focal hyalinosis and basophilic degeneration of tubular epithelial cells) (Medinsky and Irons 1985). Renal effects were also reported in B6C3F1 mice in this study; however, due to limitations in the information reported, neither a NOAEL nor a LOAEL could be determined. The study authors indicated that degenerative changes were observed in the renal tubular epithelium of "a small number of mice and were less severe than those described for CD male rats," and that males exposed to 35.8 ppm exhibited this finding. The report did not tabulate these results or report quantitative results. It should be noted that the highest exposure level of 124.5 ppm was also lethal to all mice before the end of the planned exposure period.

Using the same three animal models exposed to nitrobenzene at 5–48.7 ppm for 90 days, Hamm et al. (1984) observed dose-related renal lesions, characterized only as nephrosis, in both rat strains but not in mice. Male F344 rats appeared to be slightly more susceptible, with significant increases in the incidence of nephrosis occurring at concentrations ≥5 ppm, compared with 48.7 ppm in female F344 rats and male CD rats. Female CD rats did not exhibit this finding at any concentration (Hamm et al. 1984). The only group of rats that exhibited a treatment-related change in kidney weight was male CD rats exposed to 48.7 ppm; in this group, a significant increase in kidney weight was noted (Hamm et al. 1984). Neither male nor female B6C3F1 mice exposed to nitrobenzene showed changes in kidney histology (Hamm et al. 1984). Male mice exposed to 47.7 ppm showed increases in absolute and relative-to-brain-weight kidney weight, but not relative-to-body-weight kidney weight.

In the chronic-duration study of nitrobenzene inhalation exposure (Cattley et al. 1994, 1995; CIIT 1993), organ weight changes in both male and female F344 rats included increased absolute and relative kidney weights at 24.8 ppm (the highest concentration tested). In F344 rats, increases in renal tubular hyperplasia, cysts (in males) and chronic nephropathy (females) were reported at 24.8 ppm, as were increases in renal tubular suppurative inflammation in both sexes. Male CD rats showed no significant changes in kidney weights at any exposure level; the only renal effect observed in these animals was mineralization at the highest concentration of 24.8 ppm. CIIT (1993) noted, however, that the male CD rats in the highest exposure group exhibited changes associated with secondary hyperparathyroidism (mineralization and fibrous osteodystrophy in various organs) that suggested that the severity of nephropathy was increased by nitrobenzene treatment (without a change in incidence of the finding).

In female mice, absolute and relative kidney weights were increased at the highest exposure level (49.1 ppm); no biologically relevant organ weight changes were observed in male mice (Cattley et al. 1994, 1995; CIIT 1993). Male, but not female mice showed a higher incidence of kidney cysts at 49.1 ppm (Cattley et al. 1994, 1995; CIIT 1993). No other renal lesions were noted in mice.

A 14-day study of male Wistar rats exposed to nitrobenzene by gavage demonstrated renal effects at the one dose level tested, 100 mg/kg/day (Oladele et al. 2021). The findings included increases in serum urea and creatinine, as well as lesions consisting of mild fibrosis, hemorrhage, and marked glomerular shrinkage (Oladele et al. 2021). In an acute-duration immunotoxicity study that included assessment of kidney weights, female B6C3F1 mice exposed to nitrobenzene for 14 days via gavage had increases in absolute, but not relative kidney weight with 300 mg/kg/day exposure (Burns et al. 1994). There was not a significant increase in kidney weight at 100 mg/kg/day in that study (Burns et al. 1994). In addition, histopathology examination showed no renal effects in the female mice at any dose (Burns et al. 1994).

Intermediate-duration oral exposure to nitrobenzene induced significant increases in kidney weights in male Sprague-Dawley rats after 54 days of exposure to doses ≥60 mg/kg/day (Mitsumori et al. 1994) and in male and female F344 rats after 90 days of exposure to ≥9.375 and ≥75 mg/kg/day, respectively (NTP 1983a). At the same doses, male Sprague-Dawley and female F344 rats also exhibited histopathology changes that were suggestive of hemolysis (hemosiderin or pigment deposition). Additionally, NTP (1983a) reported the observation of pale green hyaline globules in renal cortical tubular cells in rats of the 75 and 150 mg/kg/day dose groups; no further description of these findings was provided. In male mice in the 90-day gavage study, kidney weights were increased at 300 mg/kg/day nitrobenzene, a dose at

which half of the male mice died prematurely (NTP 1983a). In female mice, relative (to body weight) kidney weight was significantly increased at the 300 mg/kg/day dose level, but the difference from controls was not significant for absolute kidney weight or kidney weight relative to brain weight (NTP 1983a). No microscopic kidney lesions were seen in the mice (NTP 1983a).

In studies of dermal exposure, kidney effects were observed after acute- and intermediate-duration exposures to nitrobenzene. In F344 rats exposed for 12 days, kidney weights were increased in males at 0.8 g/kg, while female rats exhibited no treatment-related change in kidney weight. Renal histopathology changes were seen only at the highest dose (1.6 g/kg), a dose that was lethal to all animals. Females, but not males, that died prematurely at this dose showed evidence of cytoplasmic vacuolation and degeneration in renal cortical tubules (NTP 1982). In B6C3F1 mice in the same study, no change in kidney weights were seen in either males or females (NTP 1982). As with rats, all mice exposed to 1.6 g/kg died or were sacrificed moribund prior to study termination, and in mice, this was the only group examined for histopathology. There were no microscopic kidney findings in this group (NTP 1982).

In the 90-day dermal application study, F344 rats exposed to 0.4 g/kg (the highest dose at which any rats survived to study termination) exhibited congestion in the kidneys (NTP 1983b). Kidney weights were not altered by exposure in female rats, but there were small increases in relative (to body weight) kidney weights in males exposed to 0.2 and 0.4 g/kg (NTP 1983b). Neither absolute kidney weights nor kidney weights relative to brain weight were altered at either of these doses in the male rats (NTP 1983b). Male mice exposed to nitrobenzene by dermal application exhibited dose-related increases in kidney weights at ≥0.2 g/kg (NTP 1983b). No changes to kidney weight were seen at doses up to 0.4 g/kg in female mice (the highest dose of 0.8 g/kg resulted in deaths of nearly all mice prior to study termination). No treatment-related renal lesions were observed in the mice (NTP 1983b).

Mechanisms. Nitrobenzene induced oxidative stress in the kidneys of rats given 100 mg/kg/day nitrobenzene by gavage for 14 days (Oladele et al. 2021). The study authors reported increases in kidney levels of malondialdehyde, nitric oxide, myeloperoxidase, and hydrogen peroxide along with concurrent reductions in renal concentrations of reduced glutathione, superoxide dismutase, and catalase at the end of the exposure period. No other data on potential mechanisms of nitrobenzene-induced kidney changes were located.

2.11 DERMAL

No human studies that assessed the dermal toxicity of nitrobenzene from inhalation, oral, or dermal exposure were located. Additionally, no studies were located that described any dermal effects in experimental animals after inhalation or oral ingestion of nitrobenzene. Studies in which nitrobenzene was applied directly to the skin of the animal did not report significant dermal effects (NTP 1982, 1983b).

2.12 OCULAR

No human studies have been located that assessed the ocular toxicity of nitrobenzene from inhalation, oral, or dermal exposure. Additionally, no studies were located that described any ocular effects after oral ingestion or dermal exposure to nitrobenzene in experimental animals.

Slight corneal clouding was observed in Crl:CD rats after 4 hours of inhalation exposure at a concentration of 514 ppm in an acute lethality study (Dupont 1981).

2.13 ENDOCRINE

No studies were located that evaluated the endocrine effects from nitrobenzene exposure in humans by any route of exposure.

In experimental animal studies, effects on the adrenal glands have been observed in F344 and CD rats exposed by inhalation (Hamm et al. 1984), and in mice exposed by inhalation (Hamm et al. 1984; Cattley et al. 1994, 1995; CIIT 1993), oral (NTP 1983a), and dermal (NTP 1983b) exposure routes. Both male and female F344 rats and male CD rats exhibited increased basophilia of adrenal medullary cells after 90 days of exposure to 48.7 ppm (Hamm et al. 1984), but no adrenal effects were observed after 2 years of exposure to nitrobenzene concentrations up to 24.8 ppm. Female mice exhibited adrenal gland cortical cell vacuolization or fatty change after 90 days of inhalation exposure to ≥5 ppm (Hamm et al. 1984), after 2 years of inhalation exposure to ≥24.8 ppm (Cattley et al. 1994, 1995; CIIT 1993), after 90 days of oral exposure to 300 mg/kg/day (NTP 1983a), and after 90 days of dermal exposure to ≥0.05 g/kg (NTP 1983b). Male mice did not show evidence of nitrobenzene-induced effects on the adrenal glands.

Thyroid changes associated with nitrobenzene exposure were observed in male CD rats and male B6C3F1 mice exposed by inhalation. Slight thyroid follicular cell hypertrophy was noted in male CD rats after

90 days of exposure to 48.7 ppm nitrobenzene (Hamm et al. 1984). Male mice exhibited thyroid follicular cell hyperplasia after 2 years of exposure to concentrations ≥24.8 ppm (Cattley et al. 1994, 1995; CIIT 1993). No thyroid effects were noted in male or female F344 rats, female CD rats, or female B6C3F1 mice exposed to nitrobenzene by inhalation (Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984).

Chronic-duration inhalation exposure to nitrobenzene resulted in microscopic changes in the pancreas of female B6C3F1 mice and the parathyroid gland of male F344 rats. In female mice exposed to 49.1 ppm nitrobenzene for 2 years, an increase in the incidence of mononuclear cell infiltrate in the pancreas was observed (Cattley et al. 1994, 1995; CIIT 1993). Male F344 rats showed an increase in the incidence of diffuse hyperplasia of the parathyroid gland after exposure to 24.8 ppm nitrobenzene (Cattley et al. 1994, 1995; CIIT 1993). There were no effects on the pancreas or parathyroid gland noted in female F344 rats, female CD rats, or female B6C3F1 mice exposed to nitrobenzene by inhalation (Cattley et al. 1994, 1995; CIIT 1993; Hamm et al. 1984) or in rats or mice exposed by oral or dermal routes (NTP 1983a, 1983b).

In an acute-duration oral study of nitrobenzene in male Wistar rats, exposure to 100 mg/kg/day for 14 days resulted in decreased serum levels of thyroid-stimulating hormone (TSH); other thyroid hormone levels or related endpoints were not evaluated in this study (Oladele et al. 2020a). No other thyroid effects were observed in rats or mice exposed by oral or dermal routes (NTP 1983a, 1983b).

Mechanisms. Data on potential mechanisms of the adrenal, thyroid, and parathyroid effects of nitrobenzene are limited to a single study of oxidative stress in thyroid cells. In an *in vitro* study using porcine thyroid cells, Zasada and Karbownik-Lewinska (2015) showed that exposure to nitrobenzene resulted in concentration-related increases in lipid peroxidation measured as malondialdehyde and 4-hydroxy alkenals. Concurrent treatment with antioxidants (melatonin and propylthiouracil) mitigated the nitrobenzene effects on oxidative stress (Zasada and Karbownik-Lewinska 2015).

2.14 IMMUNOLOGICAL

No studies were located that examined potential immunologic effects of nitrobenzene exposure on humans via any route of exposure.

After intermediate-duration inhalation exposure to nitrobenzene, proliferative changes in the lymph nodes were seen in F344 and CD rats, but not in mice (Hamm et al. 1984). These findings were described as

proliferation of plasma cells containing mitotic figures and extending as clusters or sheets from the subcapsular sinusoids, sometimes in conjunction with increased numbers of mast cells and macrophage infiltration at the margins. The incidences of these findings did not reach statistical significance (compared with control incidence) at any exposure level. In a chronic-duration inhalation study, involution of the thymus occurred at increased incidence in female B6C3F1 mice exposed to 49.1 ppm nitrobenzene (Cattley et al. 1994, 1995; CIIT 1993). There were no changes in the thymus of male mice or in rats in the same study exposed to concentrations up to 24.5 ppm nitrobenzene (Cattley et al. 1994, 1995; CIIT 1993). The thymus is known to shrink with age, so this finding should be interpreted within that context (Aspinall and Andrew 2000).

Only one study of nitrobenzene specifically focused on assessment of immune system effects. In this acute-duration oral exposure study (Burns et al. 1994), female B6C3F1 mice were administered 0, 30, 100, or 300 mg/kg of nitrobenzene in corn oil via gavage for 14 days in several different experiments. Thymus weight was measured, and the following immune function assays were assessed: spleen IgM and IgG antibody response following stimulation with sheep erythrocytes (sRBC), spleen cell proliferation following stimulation with mitogens, mixed leukocyte response to allogeneic spleen cells, delayed hypersensitivity response to keyhole limpet hemocyanin (KLH), measurement of serum complement proteins, reticuloendothelial system clearance of sRBCs, peritoneal cell number count, macrophage phagocytic activity, natural killer cell activity, and host resistance to various microbes and a tumor cell line.

There were mortalities (8.5% of animals) among the mice across several experiments at the 300 mg/kg/day dose (Burns et al. 1994). No treatment-related changes in thymus weight were observed, and differential leukocyte counts in peripheral blood were unchanged with the exception of a significant dose-related trend for decrease in eosinophil count. Nitrobenzene treatment did not significantly affect serum complement levels or delayed hypersensitivity response (Burns et al. 1994).

In the sRBC assay performed 4 days after the end of nitrobenzene exposure, there were significant increases in spleen weight and spleen cell number at 300 mg/kg/day and significantly reduced numbers of IgM antibody-forming cells (AFCs) at ≥100 mg/kg/day. On the 5th day after exposure, there were significant increases in spleen weight and spleen cell number at ≥100 mg/kg/day. No effect of treatment on IgG AFCs was detected. The effects on spleen weight and IgM AFC counts did not persist after a 20-day recovery period.

The spleen cell mitogenic response assay was confounded by an increase in background (in the absence of mitogen) 3H-thymidine incorporation induced by nitrobenzene doses of 100 and 300 mg/kg/day, evident also when measured as spleen cell number (Burns et al. 1994). When expressed as ^{3}H-thymidine incorporation, T-cell mitogen (phytohemagglutinin and concanavalin A) response appeared to be suppressed at ≥100 mg/kg/day nitrobenzene and there was no effect of treatment on B-cell mitogen (lipopolysaccharide) response. However, when expressed as stimulation indices, T-cell mitogenic response was stimulated and B-cell mitogenic response was inhibited at doses ≥100 mg/kg/day (Burns et al. 1994). The mixed lymphocyte response to allogeneic spleen cells from DBA/2 mice was suppressed with nitrobenzene exposure; however, this assay was also influenced by the background spleen cell proliferation induced by nitrobenzene. When expressed as stimulation index, a decrease was observed at ≥100 mg/kg/day, indicating that nitrobenzene reduced the ability of splenic T cells to recognize and respond to alloantigens. The ratio of responding and nonresponding cells in the spleen was likely altered by nitrobenzene-induced increase in numbers of non-immune cells. Finally, natural killer cell activity in the spleen, measured as the cells' ability to lyse the YAC-1 cell *in vitro* was decreased at ≥100 mg/kg/day (Burns et al. 1994). The study authors suggested that the percentage of natural killer cells in the spleen could have decreased due to increases in other cell types in the spleen.

Measurement of peritoneal cell numbers and their macrophage function showed increases in both at 300 mg/kg/day nitrobenzene (Burns et al. 1994). In assays of radiolabelled sRBC clearance by the fixed mononuclear phagocyte system, overall phagocytic activity was increased with dose, and macrophage activity was increased in the liver at 300 mg/kg/day and decreased in the spleen and lungs at ≥100 mg/kg/day. The study authors suggested that the decreases in spleen and lungs resulted from the increases in uptake by the liver (fewer particles available for uptake by other organs), and that the increased hepatic uptake was partly attributable to liver enlargement (Burns et al. 1994).

The host-resistance assays showed little effect of nitrobenzene, except when the mice were challenged with *Listeria monocytogenes* (Burns et al. 1994). Exposed mice were more susceptible (e.g., exhibited higher mortality) to *L. monocytogenes* at 100 and 300 mg/kg/day nitrobenzene. The study authors noted that resistance to *L. monocytogenes* is mediated by T-lymphocytes, macrophages, and complement activity (Burns et al. 1994).

Other studies provide limited data on immune system endpoints. F344 rats treated by gavage for 90 days showed dose-related increases in the incidence of lymphoid depletion in the spleen at doses ≥37.5 (males) and ≥18.75 mg/kg/day (females) (NTP 1983a). In female mice exposed by gavage for 90 days, lymphoid

depletion in the spleen was observed at doses ≥150 mg/kg/day (NTP 1983a). Only one male mouse at the 300 mg/kg/day dose exhibited this effect.

Lymphoid atrophy in the spleen was also seen in acute- and intermediate-duration studies of rats exposed by dermal application at doses ≥0.4 and 0.2 g/kg, respectively (NTP 1982, 1983b). Mice exposed dermally to nitrobenzene did not show this change. However, mice exposed to 0.8 g/kg (a dose that caused premature deaths in 9/10 males and 8/10 females) for 90 days by skin application had thymic atrophy with marked depletion of lymphocytes (NTP 1983b).

2.15 NEUROLOGICAL

Nitrobenzene has shown to induce neurological effects in humans and animals. Neurological effects were noted in the case of a woman who was occupationally exposed to nitrobenzene for 17 months at an unknown level. These effects included headache, nausea, vertigo, confusion, and paresthesia (Ikeda and Kita 1964). Similar effects were reported in case studies of acute oral ingestion of nitrobenzene (Carter 1936; Leader 1932; Myslak et al. 1971). Lesions in the brain (corpus callosum, centrum semiovale, dentate nuclei, and/or substantia nigra) have been observed in humans after accidental or intentional nitrobenzene ingestion (Dsouza et al. 2022; Kumar et al. 2017; Boukobza et al. 2015). In a case study of a woman who died by suicide after consumption of nitrobenzene, petechial hemorrhages were found in both cerebral hemispheres (Gupta et al. 2012). Levels of nitrobenzene associated with these effects were not reported in these studies. No studies on the effects on the neurological system in humans after dermal exposure were located.

In an acute lethality study, 4-hour exposure of male CRL:CD rats (Dupont 1981) to 439 ppm nitrobenzene resulted in hyperactive and aggressive behavior several days after exposure; at 555 ppm, rats exhibited tremors 1–2 days after exposure, and 6/10 rats died during this time frame. When CD rats and B6C3Fl mice were exposed to nitrobenzene at 124.5 ppm daily for 2 weeks, damage to the hindbrain (cerebellar peduncle), including bilateral cerebellar perivascular hemorrhage and malacia (cell breakdown), was observed in 8/19 mice (both sexes) and in 14/19 rats (both sexes) (Medinsky and Irons 1985). No brain lesions were found in F344 rats exposed to the same levels. The reason for these strain differences under similar conditions is not apparent. In the 90-day inhalation study, no neurologic signs of toxicity, alterations in brain weight, or brain histopathology changes were observed in B6C3F1 mice or F344 or CD rats exposed to concentrations up to 48.7 ppm nitrobenzene in air (Hamm et al. 1984).

Likewise, there were no effects on these parameters in rats and mice exposed to concentrations up to 24.8 and 49.1 ppm, respectively, for 2 years (Cattley et al. 1994, 1995; CIIT 1993).

A single gavage dose of 550 mg/kg nitrobenzene induced moderate to severe ataxia, loss of righting reflex, and lack of responsiveness to stimuli in male F344 rats (Morgan et al. 1985). At necropsy, these animals exhibited hemorrhages in the brain stem and cerebellum, and bilateral symmetric degeneration in the cerebellum and cerebellar peduncles (Morgan et al. 1985). Oladele et al. (2020b) evaluated neurobehavioral effects and brain histopathology in Wistar rats given 100 mg/kg/day nitrobenzene by daily gavage for 14 days. The exposed rats exhibited decreased exploratory behavior and increased defecation, and the study authors reported degenerative lesions in the cerebellum, cerebrum, and hippocampus. In addition, the study authors observed increased acetylcholinesterase activity and decreased dopamine levels in the brain (Oladele et al. 2020a). In a 2-week study of immune system effects, mice exposed to 300 mg/kg/day exhibited clinical signs of neurotoxicity including ataxia, circling behavior, and lethargy; at the same dose, there were premature mortalities related to treatment (Burns et al. 1994).

With oral exposure to 60 mg/kg/day in a repeat-dose and reproductive/developmental toxicity screening study, one female Sprague-Dawley rat experienced torticollis (a condition in which the neck muscles are contracted causing the head to tilt to one side) and abnormal gait during lactation (after about 50 days of exposure) (Mitsumori et al. 1994). Additionally, in this same study at 60 and 100 mg/kg/day, neuronal necrosis/gliosis in certain nuclei in the cerebellar medulla and pons were observed in 3/10 and 10/10 males, respectively (Mitsumori et al. 1994). Histopathology was not examined in the female rats in this study (Mitsumori et al. 1994).

Oral exposure to nitrobenzene for 90 days also resulted in clinical signs and neuropathology in rats and mice. Ataxia was observed in all female F344 rats exposed to 75 mg/kg/day (NTP 1983a). Male rats exposed to the highest dose of 150 mg/kg/day exhibited ataxia, lethargy, trembling, circling, and head tilt; at this dose, 9/10 males (along with 3/10 females) died before the end of the exposure period (NTP 1983a). It should be noted that all rats receiving doses of 75 and 150 mg/kg/day were cyanotic, which may have contributed to, and/or account for, the ataxia and other clinical signs. Brain weights were not affected by nitrobenzene exposure in rats, but histopathology changes consisting of brain stem hemorrhage, degeneration, and malacia were noted to occur at higher incidence (NTP 1983a). In males, hemorrhages were observed in the brain stem in 1/10, 4/10, 4/10, 5/10, and 2/10 animals exposed to 9.375, 18.75, 37.5, 75, and 150 mg/kg/day (respectively), but not in any control males. In female rats, a

higher incidence of brain stem hemorrhage was observed only in the 150 mg/kg/day group (7/10 versus 4/10 controls). At 150 mg/kg/day, both male and female rats exhibited degeneration and malacia in the brain stem (4/10 males and 3/10 or 4/10 females, compared with 0/10 controls of each sex). Other neuropathology findings in rats exposed to 150 mg/kg included degenerative changes in the pons, dentate nucleus, and cerebellar peduncle/olivary nucleus. These changes were described as spongy degeneration, necrosis, karyorrhexis, neutrophils, lymphocytes, plasma cells, and fusiform cells (NTP 1983a).

Mice were somewhat less sensitive to the neurological effects of nitrobenzene in the 90-day gavage study (NTP 1983a). Ataxia was observed at 300 mg/kg/day dose in 9/10 male and 1/10 female mice; three male mice (and no females) at this dose died prematurely (NTP 1983a). Females at this dose were characterized as irritable, and one female showed other neurological signs including hyperactivity and head bobbing. At 75 mg/kg/day, one male mouse was lethargic. Mice did not show cyanosis. There were no changes in brain weights and no increases in the incidences of histopathology findings in the brains of mice (NTP 1983a).

In the 12-day study of dermal exposure, all rats and mice died or were sacrificed moribund prior to study termination at doses ≥1.6 g/kg; ataxia, prostration, and dyspnea were seen in these animals prior to death (NTP 1982). Cyanosis was observed in all male and female rats by day 2 of treatment with ≥0.8 g/kg and in 2/5 male rats by day 8 of treatment with 0.4 g/kg (NTP 1982). At 0.8 g/kg, male Fischer F344 rats also displayed inactivity, possibly a consequence of hypoxia. No cyanosis or clinical signs of toxicity were noted in male rats at the lowest dose of 0.2 g/kg or in female rats treated with 0.2 or 0.4 g/kg (NTP 1982). The only histopathology findings in rats were small foci of hemorrhage in the cerebral and cerebellar cortex in 4/5 male and 5/5 female rats at the 1.6 g/kg dose (NTP 1982). Mice in this study did not show cyanosis at any dose, or clinical signs of neurotoxicity at doses that were not lethal (≤0.8 g/kg) (NTP 1982). As with the rats, 2/5 male mice and 1/5 female mice displayed small foci of hemorrhage in the cerebral and cerebellar cortex after dermal exposure to 1.6 g/kg nitrobenzene (NTP 1982).

In the follow-up 90-day dermal exposure study, all rats and 17/20 mice died prematurely with exposure to 0.8 g/kg nitrobenzene (NTP 1983b). Male and female rats displayed cyanosis, lethargy, and ataxia with exposure at this dose (NTP 1983b). Brain and brain stem hemorrhages were observed at low incidence in all groups of males including controls. At the highest dose of 0.8 g/kg, male and female rats exhibited effects in the cerebral and/or cerebellar white matter and the pons, including necrosis, vacuolization, and/or degeneration. Malacia was observed in the brain stem and pons in males and females at this dose. Control female rats showed no microscopic lesions in the brain. While there were no clear dose-related

trends for individual brain lesions in the treated female rats, when the reported lesions (brain and brain stem hemorrhages and vacuolization in the brain stem or cerebral or cerebellar white matter) were considered together, effects in the female rats occurred at doses as low as 0.1 g/kg (NTP 1983b). As was seen in the acute-duration study (NTP 1982), mice were less sensitive than rats to neurological effects of 90-day dermal exposure to nitrobenzene. Mice were not cyanotic at any dose, but did exhibit clinical signs including hypoactivity, circling, and head tilting or leaning with exposure to 0.8 g/kg nitrobenzene (NTP 1983b). There were no effects of treatment on brain weight or histopathology in mice (NTP 1983b).

Mechanisms. Some, but not all neurological effects may be mediated by hypoxia resulting from methemoglobinemia and/or erythrocyte hemolysis. As noted above, rats exhibiting neurological signs of toxicity were often cyanotic, while mice were not. There are few other data pertaining to neurotoxicity mechanisms of nitrobenzene. In the 14-day gavage study in Wistar rats described above, Oladele et al. (2020a) also showed that nitrobenzene exposure (100 mg/kg/day) increased oxidative stress in the cerebrum, mid brain, and cerebellum, as shown by statistically significant increases in malondialdehyde and hydrogen peroxide levels and decreases in levels of superoxide dismutase, reduced glutathione, and catalase. There is inconsistent information on the interaction between nitrobenzene and acetylcholinesterase activity. In cell-free experiments, nitrobenzene exhibited weak inhibition of acetylcholinesterase activity (Chen et al. 2019); however, in rats, oral exposure to 100 mg/kg/day nitrobenzene resulted in a statistically significant increase in brain acetylcholinesterase activity (Oladele et al. 2020a).

2.16 REPRODUCTIVE

No studies were located regarding reproductive effects in humans following inhalation, oral, or dermal exposure to nitrobenzene. In experimental animals, nitrobenzene is a known testicular toxicant and has been used as a positive control in many studies aiming to evaluate toxic effects on spermatogenesis (Allenby et al. 1990, 1991; Linder et al. 1992). However, the evidence that nitrobenzene affects female reproduction in experimental animals is limited.

Testicular Effects. Evidence of testicular toxicity is seen in acute-, intermediate-, and chronic-duration studies of nitrobenzene exposure of rats and mice via inhalation, oral, and dermal routes. For example, in an acute-duration study in Fischer 344 rats, decreases in testicular weight and size were observed in rats exposed to 124.5 ppm for 2 weeks via inhalation (Medinsky and Irons 1985). Further, testicular lesions

were also observed in these rats, including an increase in multinucleated giant cells, Sertoli cell hyperplasia, interstitial edema, and severe dysfunctional spermiogenesis. Few sperm, with arrested maturation at primary and secondary spermatocyte stages, were present in seminiferous tubules, and the lumen of the ductus epididymis contained a reduced number of sperm. After a 2-week recovery period, the lesions were still present, although the Sertoli cell hyperplasia and the increased numbers of multinucleated giant cells were less severe (Medinsky and Irons 1985). At the same exposure concentration, male CD rats and B6C3F1 mice exhibited similar effects. In addition, male mice showed testicular degeneration and increased multinucleated giant cells at 35.8 ppm (Medinsky and Irons 1985).

Hamm et al. (1984) reported that both F344 and Sprague-Dawley rats exposed to nitrobenzene at 50 ppm for 90 days had testicular atrophy, bilateral degeneration of the seminiferous tubules, and a reduction in, or absence of, mature sperm in the epididymis. The lesions were more severe in Sprague-Dawley rats, which exhibited complete degeneration of the epithelium in seminiferous tubules. No testicular lesions were observed in B6C3Fl mice under the same exposure conditions (Hamm et al. 1984). In chronic-duration inhalation experiments conducted by Cattley et al. (1994, 1995; CIIT 1993), testicular lesions (bilateral atrophy of the testes and epididymal hypospermia) were observed in male CD rats at the highest exposure concentration (24.8 ppm) and epididymal hypospermia was observed in male mice at the highest concentration tested in that species (49.1 ppm) (Cattley et al. 1994, 1995; CIIT 1993).

Acute- and intermediate-duration oral studies in experimental animals provide additional evidence for testicular effects of nitrobenzene. In rats, single gavage doses of 250–300 mg/kg nitrobenzene resulted in decreased testicular weights, degeneration of seminiferous epithelium, sloughing of cells into the tubular lumen, loss of mature spermatids, increased numbers of multinucleated giant cells, decreased sperm counts, and abnormal sperm morphology (Levin et al. 1988; Linder et al. 1992; McLaren et al. 1993a). When nitrobenzene doses of 60 mg/kg/day were administered by gavage for 3 days, no testicular effects were seen (Kawaguchi et al. 2004), but with 14 days of exposure at this dose, testes and epididymal weights were decreased, as were sperm counts and motility (Iida et al. 1997; Kawashima et al. 1995). Two weeks of exposure by gavage to doses of 100 mg/kg/day reduced testicular and epididymal weights and also resulted in decreases in serum hormone levels (testosterone, prolactin, luteinizing hormone, and follicle stimulating hormone) in Wistar rats (Oladele et al. 2020c).

With intermediate-duration (21–90 days) oral exposures to ≥40 mg/kg/day, similar but more severe testicular effects were observed in rats (Iida et al. 1997; Kato et al. 2002; Kawaguchi et al. 2004; Kawashima et al. 1995; Mitsumori et al. 1994; NTP 1983a). As part of a study examining a new method

to evaluate mitochondrial function in sperm, mature male Sprague-Dawley rats were given nitrobenzene by daily gavage for 49 days before sperm were collected for analysis of motility (Kato et al. 2002). In the groups exposed to 40 and 60 mg/kg/day, there were no motile sperm; exposure to 20 mg/kg/day did not alter sperm motility (Kato et al. 2002). In the two rat studies that examined more than one dose level, isolated occurrences of seminiferous tubule atrophy were seen at doses of 20–37.5 mg/kg/day (Mitsumori et al. 1994; NTP 1983a). Only one intermediate-duration oral study in mice included examination of the testes. NTP (1983a) observed testicular atrophy in mice at 150 mg/kg/day, but not 75 mg/kg/day, after 90 days of treatment. At the highest dose in this study (300 mg/kg/day), half of the male mice died or were sacrificed moribund prior to termination (NTP 1983a).

In the two studies that evaluated nitrobenzene toxicity after dermal exposure, effects on the testes similar to those seen in the inhalation and oral studies were observed. After 12 days of dermal exposure to 0.8 g/kg, rats exhibited atrophy of the seminiferous tubules, absence of spermatids and spermatozoa, increased multinucleated giant cells, and decreased testes weight, while these effects were not seen in mice at the same doses (NTP1982). After 90 days of dermal exposure, similar but more severe testicular effects were seen at 0.4 g/kg in rats, while mice exhibited slightly less severe changes, including decreased testes weight, testicular atrophy, and hypospermatogenesis, at the same dose (NTP 1983b). Sloughing of spermatocytes, spermatids, and spermatozoa into the tubular lamina was reported in a few mice (incidence not reported) in the group dosed with 0.2 g/kg (NTP 1983b).

Other Reproductive Effects. Intermediate-duration (90 days) oral exposure to nitrobenzene resulted in uterine atrophy in two female rats given doses of 150 mg/kg/day (NTP 1983a). In addition, after 90 days of exposure via dermal application at 0.8 g/kg, 6/10 female rats and 5/10 female mice displayed atrophy of the uterus (NTP 1983b). It should be noted that all female rats and 8/10 female mice died before the end of the exposure period at this dose (0.8 g/kg). The study authors also reported that the ovaries of a few rats were congested (NTP 1983b); however, the exact number of rats experiencing this effect was not stated.

In a 2-generation inhalation study in Sprague-Dawley rats, 10 weeks of nitrobenzene exposure resulted in a decrease in fertility indices at 40 ppm for F0 and F1 generations, while other reproductive parameters were unaltered (Dodd et al. 1987). The study data suggested that the decrease in fertility was caused by males. Dodd et al. (1987) observed atrophy of seminiferous tubules, spermatocyte degeneration, and reduced testicular and epididymal weights in the F0 and F1 generations with 40 ppm exposure. When pregnant rats were exposed by inhalation to nitrobenzene concentrations up to 39.4 ppm on GDs 6–15 and New Zealand white rabbits were exposed to concentrations up to 104 ppm on GDs 7–19, there were no

significant adverse effects on numbers of corpora lutea, implantations, or resorptions (Biodynamics 1983, 1984; Tyl et al. 1987).

Despite the effects on testicular weight, sperm count, and sperm motility, oral exposure of male rats to 60 mg/kg/day nitrobenzene by gavage for 14 days did not affect copulation or fertility rates (Kawashima et al. 1995). However, when the same dose was administered for 21 or 28 days, the fertility index decreased to <20 and 0%, respectively (Kawashima et al. 1995). In the repeat-dose and reproductive/ developmental toxicity screening study reported by Mitsumori et al. (1994), male and female Sprague-Dawley rats were exposed from pre-mating through lactation day 4 (for a total of 54 days). Mitsumori et al. (1994) did not observe any differences in fertility or copulation indices at any dose (up to 100 mg/kg/day), although significant testicular toxicity was demonstrated at ≥60 mg/kg/day. Because the rats were only exposed for 14 days before mating, the lack of effect on fertility is not unexpected based on the findings of Kawashima et al. (1995); in fact, the study authors recommended a pre-mating treatment period longer than 14 days to detect effects on fertility. Testicular histopathology was not evaluated until the animals had been exposed for 54 days in the study by Mitsumori et al. (1994).

Mechanisms. The mechanism(s) by which nitrobenzene induces testicular toxicity and effects on spermatogenesis has not been fully elucidated, but may involve oxidative stress, induction of apoptosis in germ cells, and/or effects on Sertoli cell secretion of hormones or growth factors needed for germ cell differentiation and survival. Oladele et al. (2020c) observed increased lipid peroxidation and decreased levels of reduced glutathione in the testes of rats given 100 mg/kg/day nitrobenzene by daily gavage for 14 days. With this exposure regimen, the rats also exhibited decreased testicular and epididymal weights, as well as atrophic and degenerated seminiferous tubules (Oladele et al. 2020c). Using a cell-free system, Ohkuma and Kawanishi (1999) showed that nitrosobenzene, a metabolite of nitrobenzene, may cause oxidative DNA damage when this metabolite is reduced by reduced nicotinamide adenine dinucleotide (NADH) in the presence of Cu(II). The study authors suggested that the germ cell epithelium of the testis is exquisitely sensitive to oxidative DNA damage due to the continuous proliferation occurring in that tissue, and that oxidative DNA damage may be involved in the mechanism of nitrobenzene's effects on sperm production (Ohkuma and Kawanishi 1999).

Shinoda et al. (1998) exposed adult male Sprague-Dawley rats to single oral doses of nitrobenzene (250 mg/kg) and assessed DNA fragmentation in the testes at various times after dosing. The study authors observed DNA fragmentation using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) in late pachytene spermatocytes of exposed animals beginning 24 hours after dosing,

and these were in the same location where degenerating spermatocytes were observed upon microscopic examination. DNA laddering was observed (using gel electrophoresis), and electron micrographs of the degenerating spermatocytes showed nuclear chromatin condensation with crowding of cytoplasmic constituents; these findings are indicative of apoptosis. Shinoda et al. (1998) suggested that nitrobenzene could alter the secretion of Sertoli cell factors, leading to deficiencies in growth factors or hormones, and thus trigger apoptosis in germ cells. Using genetically modified mice that express a dysfunctional FasL protein, Richburg and Nañez (2003) showed that Fas-mediated signaling was not responsible for germ cell apoptosis induced by nitrobenzene, but that a dysfunctional Fas-signaling system increases the sensitivity of mice to germ cell apoptosis following nitrobenzene exposure.

McLaren et al. (1993b) observed decreased incorporation of 35S-methionine into newly synthesized proteins secreted by cultured seminiferous tubules obtained at different stages of spermatogenesis from rats exposed by gavage to 300 mg/kg nitrobenzene. The decrease was seen in stages VI–VIII and IX–XII, but not stages II–V, corresponding to the observed histological evidence of degeneration of pachytene spermatocytes at stages VI–XII at the same time after dosing. In experiments using seminiferous tubules from immature, late pubertal, and young adult rats cultured *in vitro* with nitrobenzene, McLaren et al. (1993b) observed age-dependent differences in the effect of nitrobenzene on protein secretions. Total 35S-methionine incorporation was decreased by nitrobenzene only in seminiferous tubules from young adult (70-day-old) rats, and not in 28- or 45-day-old rats. Secretion of individual proteins (not further identified) was also reduced by nitrobenzene, and the relationship to age varied by protein (McLaren et al. 1993b). These data suggest that nitrobenzene may affect spermatogenesis in part by reducing the secretion of hormones and growth factors needed for germ cell development and survival.

2.17 DEVELOPMENTAL

No studies were located regarding developmental effects in humans after inhalation, oral, or dermal exposure to nitrobenzene. The effects of nitrobenzene on the developing organism have been evaluated in rats and rabbits exposed by inhalation (Biodynamics 1983, 1984; Dodd et al. 1987; Tyl et al. 1987) and in rats exposed orally (Mitsumori et al. 1994).

When pregnant Sprague-Dawley rats were exposed to nitrobenzene via inhalation on GDs 6–15, there was no treatment-related effect on malformation incidence at concentrations up to 39.4 ppm (Tyl et al. 1987). The incidences of litters with variations consisting of ecchymosis (discoloration of the skin due to bleeding underneath) on the trunk and hole in the parietal bone were significantly elevated at the highest

concentration (39.4 ppm). The latter finding (hole in the parietal bone) may reflect delayed ossification (DeSesso and Scialli 2018; Fritz and Hess 1970). No increased malformations or variations were observed in offspring of New Zealand rabbits exposed to nitrobenzene concentrations up to 100 ppm on GDs 7–19 (Biodynamics 1984). In rabbits, the only effects noted on the offspring were significant decreases in the mean number of viable male fetuses in the groups exposed to 40 and 100 ppm (3.1 and 3.2 males per group, respectively, compared with 4.9 males in the control group) (Biodynamics 1984). The study authors (Biodynamics 1984) attributed the difference to an unusually high mean number of male fetuses in the control group, which consisted of 4.9 males and 3.2 females.

In a 2-generation inhalation exposure study in CD rats, Dodd et al. (1987) observed a 12% decrease in mean body weight of the F1 offspring on postnatal day (PND) 21 in the group exposed to 40 ppm nitrobenzene. The only oral study reporting assessment of developmental effects was a repeat-dose and reproductive/ developmental toxicity screening study in rats (Mitsumori et al. 1994). In this study, offspring body weights measured on PND 4 were significantly decreased in males at 20 mg/kg/day, while body weights of both male and female pups were reduced by more than 20% at 60 mg/kg/day. At 60 mg/kg/day, pup viability on PND 4 was also significantly reduced (66.9% compared with 99.1% in controls) (Mitsumori et al. 1994).

2.18 OTHER NONCANCER

Metabolic acidosis is a consequence of nitrobenzene-induced methemoglobinemia and subsequent tissue hypoxia, which triggers a switch to anaerobic respiration and accumulation of lactic acid. Metabolic acidosis was observed in the case of a 45-year-old woman who ingested about 50 mL of a solution containing 20% nitrobenzene in a suicide attempt (Shrestha et al. 2020). She was treated with hemodialysis and oral methylene blue administration, and was discharged 6 days later (Shrestha et al. 2020). No other studies were located regarding other noncancer effects of nitrobenzene exposure.

2.19 CANCER

U.S. Federal agencies and international scientific organizations have thoroughly reviewed the literature on nitrobenzene's carcinogenicity. Using a weight-of-evidence evaluation approach, EPA (2009a) has deemed nitrobenzene "likely to be carcinogenic to humans" by any route of exposure. The HHS NTP has determined that nitrobenzene is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in studies of animals (NTP 2021). IARC concluded nitrobenzene is possibly

carcinogenic in humans (Group 2B) based on inadequate evidence in humans and sufficient evidence in animals (IARC 1996, 2019).

Carreón et al. (2014) evaluated the association between bladder cancer and occupational exposure to o-toluidine, aniline, and nitrobenzene in a cohort of 1,786 workers at a rubber chemical manufacturing plant in New York. A total of 37 bladder cases were identified. Exposure was assessed using each employee's work history and assigned to exposure probability categories reflecting total exposure to the three compounds; exposure to nitrobenzene alone or as the primary contaminant was not evaluated. The standardized incidence ratios for bladder cancer were increased compared with a state-specific referent population for the highest probability of exposure, and analyses by duration and cumulative exposure indices yielded similar results. The study authors noted that measurements of exposure concentrations indicated that o-toluidine was present at the facility at higher concentrations than aniline or nitrobenzene, and that o-toluidine had been shown to be associated with increased risk of bladder cancer in other epidemiological studies (Carreón et al. 2014). For these reasons, it is not possible to ascertain the contribution of nitrobenzene exposure to the increased risk of bladder cancer in this study.

A 2-year, chronic-duration bioassay evaluated the carcinogenic effect of nitrobenzene in experimental animals (Cattley et al. 1994, 1995; CIIT 1993). Male and female B6C3F1 mice and F344 rats, and male CD rats were exposed 5 days/week, 6 hours/day to nitrobenzene via inhalation for 2 years. Mice were exposed to 0, 5, 24.8, or 49.1 ppm nitrobenzene, while rats were exposed to 0, 1, 5, or 24.8 ppm nitrobenzene. Increased incidences of liver and kidney tumors were observed in rats, and increased incidences of lung and mammary gland tumors were observed in mice. In male F344 and CD rats, significant increases in the incidences of hepatocellular adenomas and combined adenomas or carcinomas were observed at the highest exposure level (24.8 ppm). The incidences of these effects also showed statistically significant dose-related trends. In F344 rats, but not CD rats, males also exhibited significant increases in renal tubular adenomas and combined adenomas or carcinomas at 24.8 ppm, with significant dose-related trends. No increase in liver or kidney tumors was observed in female F344 rats (female CD rats were not included in this study). Female F344 rats exposed to 24.8 ppm nitrobenzene did experience a significant increase in endometrial stromal polyps, with a significant dose-related trend. Endometrial stromal polyps are noncancerous, and the relevance to humans of endometrial polyps in rats has been questioned based on differences in etiology and hormone sensitivity (Davis 2012).

Neither male nor female mice exhibited significant increases in liver tumor incidences when evaluated using pairwise comparison; however, a significant dose-related trend for increased hepatocellular

adenomas was observed in females (Cattley et al. 1994, 1995; CIIT 1993). In male B6C3F1 mice, a significant increase in the incidence of lung alveolar/bronchiolar adenoma or carcinoma was detected at all exposure concentrations (≥5 ppm), with a significant increase in adenoma incidence at ≥24.8 ppm. Female mice did not have increased incidences of lung tumors. Male (but not female) mice exposed to the highest concentration (49.1 ppm) also exhibited a significant increase in the incidence of thyroid follicular cell adenoma, with a significant dose-response by trend test. In female mice, the only statistically significant tumor finding was an increase in the incidence of mammary gland adenocarcinomas at 49.1 ppm; this tissue was not examined for tumors at lower exposure concentrations.

Apart from studies of genotoxicity (see Section 2.20), no studies exploring potential carcinogenic modes of action for nitrobenzene were located.

2.20 GENOTOXICITY

No studies were located regarding genotoxic effects in humans after exposure to nitrobenzene. Numerous studies have been published evaluating nitrobenzene's genotoxicity potential *in vitro*, summarized in Table 2-4, and *in vivo* using experimental animals, summarized in Table 2-5. The current evidence indicates that nitrobenzene is not mutagenic but does result in chromosomal aberrations and DNA damage.

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

		Results		
Species (test system)	Endpoint	With activation	Without activation	Reference
Salmonella typhimurium	Gene mutation	–	–	Anderson and Styles 1978; Assmann et al. 1997; Chiu et al. 1978; Dellarco and Prival 1989; EPA 1984a; Garner and Nutman 1977; Haworth et al. 1983; Ho et al. 1981; Mestankova et al. 2016; Shimizu et al. 1983; Vance and Levin 1984
S. typhimurium UmuC	Gene mutation	–	–	Bonnefoy et al. 2012
Human peripheral blood lymphocytes	Chromosomal aberrations	ND	+	Huang et al. 1995, 1996
Human sperm	Chromosomal aberrations	ND	-	Kamiguchi and Tateno 2002
V79 hamster lung fibroblasts	Micronuclei	ND	+	Bonacker et al. 2004

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

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
Human peripheral blood lymphocytes	Micronuclei	ND	+	Bonnefoy et al. 2012
Human kidney cells	Micronuclei	ND	+	Robbiano et al. 2004
Rat primary kidney cells	Micronuclei	ND	+	Robbiano et al. 2004
Human thyroid cells	DNA damage	ND	+	Mattioli et al. 2006
Human kidney cells	DNA damage	ND	+	Robbiano et al. 2004
Rat primary kidney cells	DNA damage	ND	+	Robbiano et al. 2004
Human hepatocytes	Unscheduled DNA synthesis	ND	–	Butterworth et al. 1989
Rat hepatocytes	Unscheduled DNA synthesis	ND	–	Butterworth et al. 1989
Human thyroid cells	Unscheduled DNA synthesis	ND	–	Mattioli et al. 2006

+ = positive result; – = negative result; DNA = deoxyribonucleic acid; ND = no data

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

Species (test system)	Endpoint	Result	Reference
F344 rat peripheral blood lymphocyte and isolated spleen lymphocyte	Sister chromatid exchange	–	Kligerman et al. 1983
Mouse bone marrow and spermatocyte	Chromosomal aberrations	+	Aly et al. 2014
Male Sprague-Dawley rats (liver, thyroid, and kidney cells)	DNA damage	+	Mattioli et al. 2006
Sprague-Dawley rats (kidney cells)	DNA damage	+	Robbiano et al. 2004
Male F344 rat hepatocytes	Unscheduled DNA synthesis	–	Mirsalis et al. 1982
Kunmig mice	DNA binding	+	Li et al. 2003a, 2003b

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

Bacterial Mutagenicity. In all *Salmonella typhimurium* strains, nitrobenzene was negative for mutagenicity, regardless of metabolic activation (Anderson and Styles 1978; Assmann et al. 1997; Bonnefoy et al. 2012; Chiu et al. 1978; Dellarco and Prival 1989; EPA 1984a; Garner and Nutman 1977; Haworth et al. 1983; Ho et al. 1981; Suzuki et al. 1983, 1987; Vance and Levin 1984). None of these studies showed a positive result for nitrobenzene (Table 2-4). Two studies (Suzuki et al. 1983, 1987) looked at nitrobenzene in combination with the co-mutagen, norharman (9H-pyrido[3,4-b] indole). Neither nitrobenzene nor norharman was mutagenic in *S. typhimurium* strains TA98 or TA100 without

metabolic activation; however, together with activation, reverse mutations were induced in the TA98 strain, but not in the TA100 strain. Further studies indicated that a nitroreductase-deficient TA98NR was negative for reverse mutations with activation and norharman, indicating that the presence of nitroreductase was required for mutagenicity (Suzuki et al. 1987).

Clastogenicity and Aneugenicity. Data on clastogenicity are mixed, with the majority of the evidence suggesting a genotoxic effect on the chromosome. Huang et al. (1995, 1996) observed increases in the percent of chromosomal aberrations in human peripheral blood lymphocytes from 12.4 to 33.2% as doses increased from 0.05 to 0.80 mmol/L. However, nitrobenzene did not induce structural chromosomal aberrations *in vitro* in human spermatozoa incubated with 500 µg/mL for 120 minutes without activation (Kamiguchi and Tateno 2002). Increased chromosomal aberrations were observed in mouse bone marrow cells and spermatocytes following *in vivo* gavage administration of 300 mg/kg nitrobenzene (Aly et al. 2014). Micronuclei were induced *in vitro* in hamster lung fibroblasts, primary human and rat kidney cells, and human peripheral lymphocytes. Bonacker et al. (2004) demonstrated the induction of micronuclei from nitrobenzene exposure using V79 hamster lung fibroblasts possibly by affecting tubulin assembly and spindle apparatus. A method to detect aneugens using antibody staining, indicated the micronucleus effects were aneugenic, as the staining was mostly kinetochore-positive. A statistically significant increase in micronuclei was observed in primary human and rat kidney cells exposed to 0.250–0.50 mM nitrobenzene (Robbiano et al. 2004). Bonnefoy et al. (2012) found 13–14% of human blood cells had micronuclei when exposed *in vitro* to 0.01–10 µg/mL concentrations of nitrobenzene. A cytogenetic analysis of lymphocytes in the peripheral blood or in splenic blood of rats exposed *in vivo* to nitrobenzene at concentrations of 5–50 ppm for 6 hours/day, 5 days/week for 21 days did not reveal an increase in sister chromatid exchange or chromosome aberrations in bone marrow (Kligerman et al. 1983). Robbiano et al. (2004) observed a dose-dependent increase in micronucleated rat kidney cells due to broken and detached chromosomes separated from the spindle apparatus in rats treated *in vivo* with 300 mg/kg nitrobenzene via gavage.

DNA Damage, Synthesis, and Binding. There is mixed evidence as to whether or not nitrobenzene may cause DNA damage, with consistent positive evidence of DNA damage and consistent negative results for unscheduled DNA synthesis. DNA damage was positive *in vitro* in human thyroid cells, human kidney cells, and rat kidney cells. Robbiano et al. (2004) observed a dose-dependent increase in the DNA fragmentation, as measured by comet assay, in primary rat and human kidney cells exposed to concentrations of 0.125–0.50 mM nitrobenzene for 48 hours. Mattioli et al. (2006) evaluated DNA fragmentation on human thyroid cells and found a dose-dependent increase in tail length and tail moment

at concentrations ranging from 1.25 to 5 mM. In an *in vivo* analysis presented in Mattioli et al. (2006), nitrobenzene induced a significant increase in DNA fragmentation in thyroid, liver, and kidney cells in Sprague-Dawley rats administered a single dose of 620 mg/kg. However, in two studies that evaluated unscheduled DNA synthesis both *in vivo* in rat hepatocytes (Mirsalis et al. 1982) and *in vitro* with human and rat cells (Butterworth et al. 1989), DNA repair response was not induced. Lastly, Li et al. (2003a, 2003b) observed that nitrobenzene can form adducts with hepatic DNA in male mice in a dose-response and time-course study. Mice were administered nitrobenzene intraperitoneally in corn oil at doses of 0.1–100 μg/kg and 10 mg/kg animals, and a dose-related increase in hepatic DNA adducts was observed at all dose levels within 2 hours of exposure (Li et al. 2003b). In the time course study, mice were administered a 4.1 μg/kg dose of nitrobenzene and animals were sacrificed between 4 hours and 21 days after exposure (Li et al. 2003b). Adducts were initially increased, followed by reduction over time.

Genotoxicity of nitrobenzene indicates that it is not mutagenic and does not induce sister chromatid exchange or unscheduled DNA synthesis but does have consistent positive results for induction of micronuclei and DNA damage. Mixed results for chromosomal aberrations and a single study on DNA binding were inconclusive.