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 chlorobenzene. 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 chlorobenzene, but may not be inclusive of the entire body of literature.

Animal inhalation studies are presented in Table 2-1 and Figure 2-2, and animal oral studies are presented in Table 2-2 and Figure 2-3; no dose-response dermal data were identified for chlorobenzene.

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 to chlorobenzene associated with cancer (Cancer Effect Levels, CELs) 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.

Human data regarding the potential health effects of chlorobenzene exposure are essentially limited to reports of clinical signs of neurotoxicity among occupationally-exposed workers and among volunteers exposed by inhalation.

As illustrated in Figure 2-1, available animal data suggest the following sensitive targets of chlorobenzene toxicity:

- **Hepatic endpoint:** Inhalation or oral exposure of animals to chlorobenzene resulted in hepatic effects such as liver weight increases and dose-related increased incidence and severity of histopathologic liver effects such as hepatocellular hypertrophy and degenerative and regenerative liver lesions.

- **Renal endpoint:** Inhalation or oral exposure of animals to chlorobenzene resulted in renal effects such as increased kidney weight and dose-related increased incidence and severity of histopathologic kidney effects such as tubular dilatation, interstitial nephritis, and degenerative and regenerative kidney lesions.

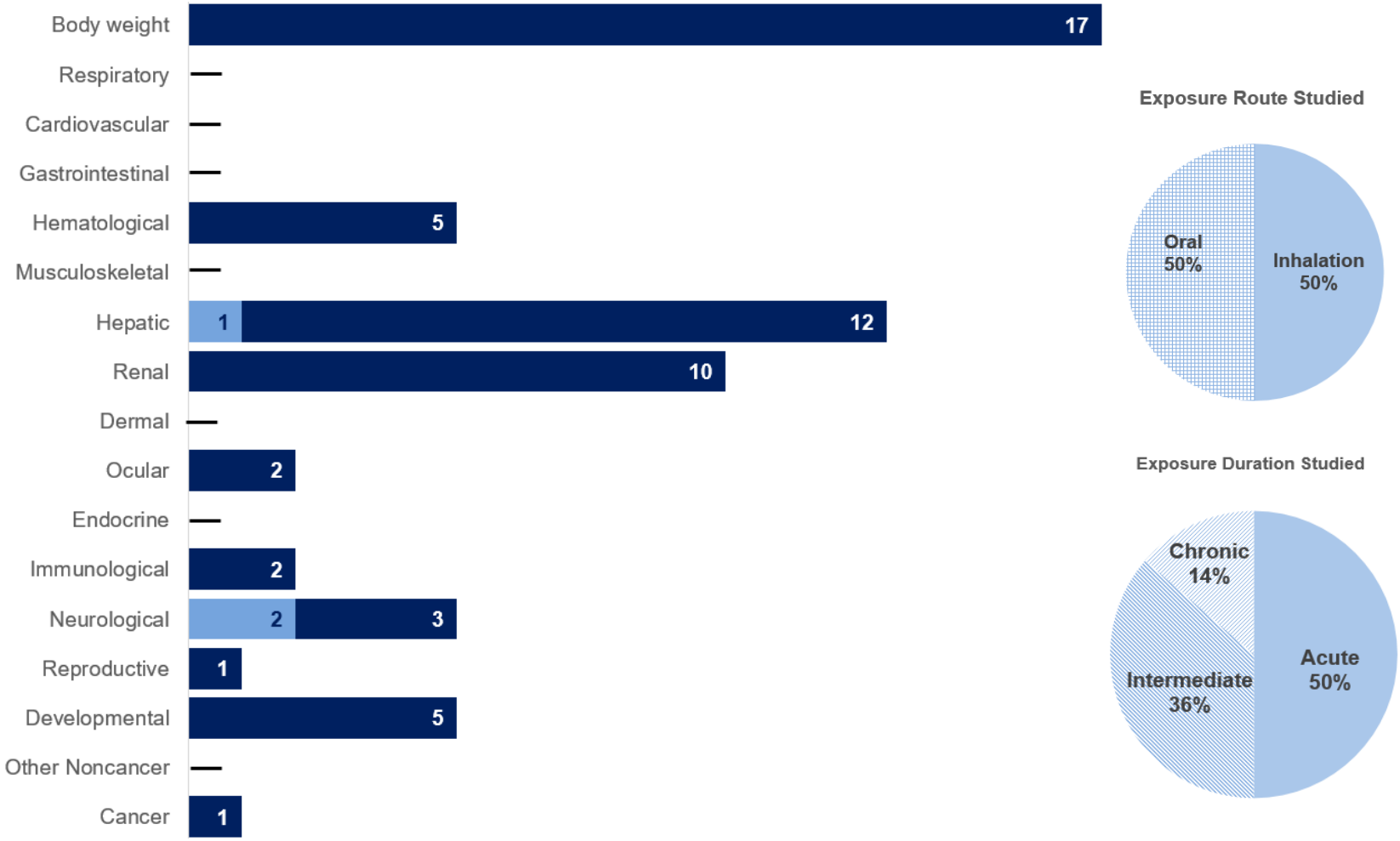
- **Neurotoxicity endpoint:** Occupational and voluntary inhalation exposure to chlorobenzene has been associated with clinical signs of neurotoxicity such as numbness, cyanosis, muscle spasms, drowsiness, headache, ocular pain, and sore throat. Neurotoxic signs in animals exposed to chlorobenzene by inhalation or gavage include ataxia, decreased activity, salivation, prostration, and narcosis.

- **Immunological endpoint:** Lymphoid depletion/necrosis in thymus and/or spleen, and myeloid depletion in bone marrow were reported among rats and/or mice in 13-week gavage studies.

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

Most studies examined the potential body weight, hepatic, and renal effects of chlorobenzene

The majority of the studies examined inhalation or oral exposure in animals; limited data were identified for humans (counts represent the number of studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 22 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

Table 2-1. Levels of Significant Exposure to Chlorobenzene – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effect
ACUTE EXPOSURE									
1	Rat (Sprague-Dawley) 5 M, 5 F	30 minutes	2,990, 5,850, 7,970	BW, CS, GN, HP, LE, OW	Bd wt	7,970			
					Hepatic	7,970			
					Renal	7,970			
					Ocular		2,990		Squinting, lacrimation
					Neuro	2,990		5,850	Ataxia, narcosis
Shell Oil Co. 1991									
2	Mouse	2 hours		LE	Death			4,300	100% mortality
Rozenbaum et al. 1947									
3	Rabbit	2 hours		LE	Death			537	
Rozenbaum et al. 1947									
4	Rat (Fischer 344) 32–33 F	GDs 6–15 6 hours/day	0, 75, 210, 590	BW, DX, FI, FX, LE, MX, OW, TG, WI	Bd wt	590			
					Develop	590			
John et al. 1984									
5	Rabbit (New Zealand white) 30 F	GDs 6–18 6 hours/day	0, 75, 210, 590	BW, DX, FI, FX, LE, MX, OW, TG, WI	Bd wt	590			
					Develop	590			
John et al. 1984									
6	Rabbit (New Zealand white) 30 F	GDs 6–18 6 hours/day	0, 10, 30, 75, 590	BW, DX, FI, FX, LE, MX, OW, TG, WI	Bd wt	590			
					Develop	590			
John et al. 1984									

Table 2-1. Levels of Significant Exposure to Chlorobenzene – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effect
7	Guinea pig (Hartley albino) 5 M, 5 F	30 minutes	2,990, 5,850, 7,970	BW, CS, GN, HP, LE, OW	Bd wt	7,970			
					Hepatic	7,970			
					Renal	7,970			
					Ocular		2,990		Squinting, lacrimation
					Neuro	2,990		5,850	Salivation, narcosis
Shell Oil Co. 1991									
INTERMEDIATE EXPOSURE									
8	Rat (Sprague-Dawley) 30 M, 30 F	2 generations 18–20 weeks per generation 6 hours/day	9, 50, 150, 450	BW, CS, FI, DX, GN, HP, LE, OF, OW	Bd wt	450			
					Hepatic	50 M 450 F	150 M		Increased mean relative liver weight, increased incidence of hepatocellular hypertrophy in parental males
					Renal	50 M 450 F	150 M		Renal lesions including chronic interstitial nephritis and foci of regenerative epithelium in parental males
					Repro	150 M 450 F	450 M		Increased incidence of degeneration of testicular germinal epithelium in the absence of apparent effects on fertility
					Develop	450			
Nair et al. 1987 (data also reported in CMA 1986)									
9	Rat (Sprague-Dawley) 32 M	Up to 24 weeks 5 days/week 7 hours/day	0, 75, 250	BC, BW, CS, EA, FI, GN, HE, HP, LE, OF, OW	Bd wt	250			
					Hemato	250			
					Hepatic	250			
NIOSH 1977									
10	Mouse (Swiss) 5 M, 5 F	3 weeks 7 hours/day	0, 543	BW, CS, HE, LE	Death			543	5/10 mice died
Zub 1978									

Table 2-1. Levels of Significant Exposure to Chlorobenzene – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effect
11	Rabbit (NS) 32 M	Up to 24 weeks 5 days/week 7 hours/day	0, 75, 250	BC, BW, CS, EA, FI, GN, HE, HP, LE, OF, OW	Bd wt	250			
					Hemato	250			
					Hepatic	250			
NIOSH 1977									
12	Dog (beagle) 6 M, 6 F	6 months 5 days/week 6 hours/day	0, 173.8, 349.8, 453.2	BC, BW, CS, EA, FI, GN, HE, HP, LE, OF, UR	Bd wt	453.2			
					Hemato	453.2			
					Hepatic	453.2			
					Renal	453.2			
Monsanto Co. 1980									

[a]The number corresponds to entries in Figure 2-2; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

BC = serum (blood) chemistry; Bd wt or BW = body weight; CS = clinical signs; EA = enzyme activity; Develop = developmental; DX = developmental toxicity; F = female(s); FI = food intake; FX = fetal toxicity; GD = gestation day(s); GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MX = maternal toxicity; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function; OW = organ weight; Repro = reproductive; TG = teratogenicity; UR = urinalysis; WI = water intake

Figure 2-2. Levels of Significant Exposure to Chlorobenzene – Inhalation

Acute (≤14 days)

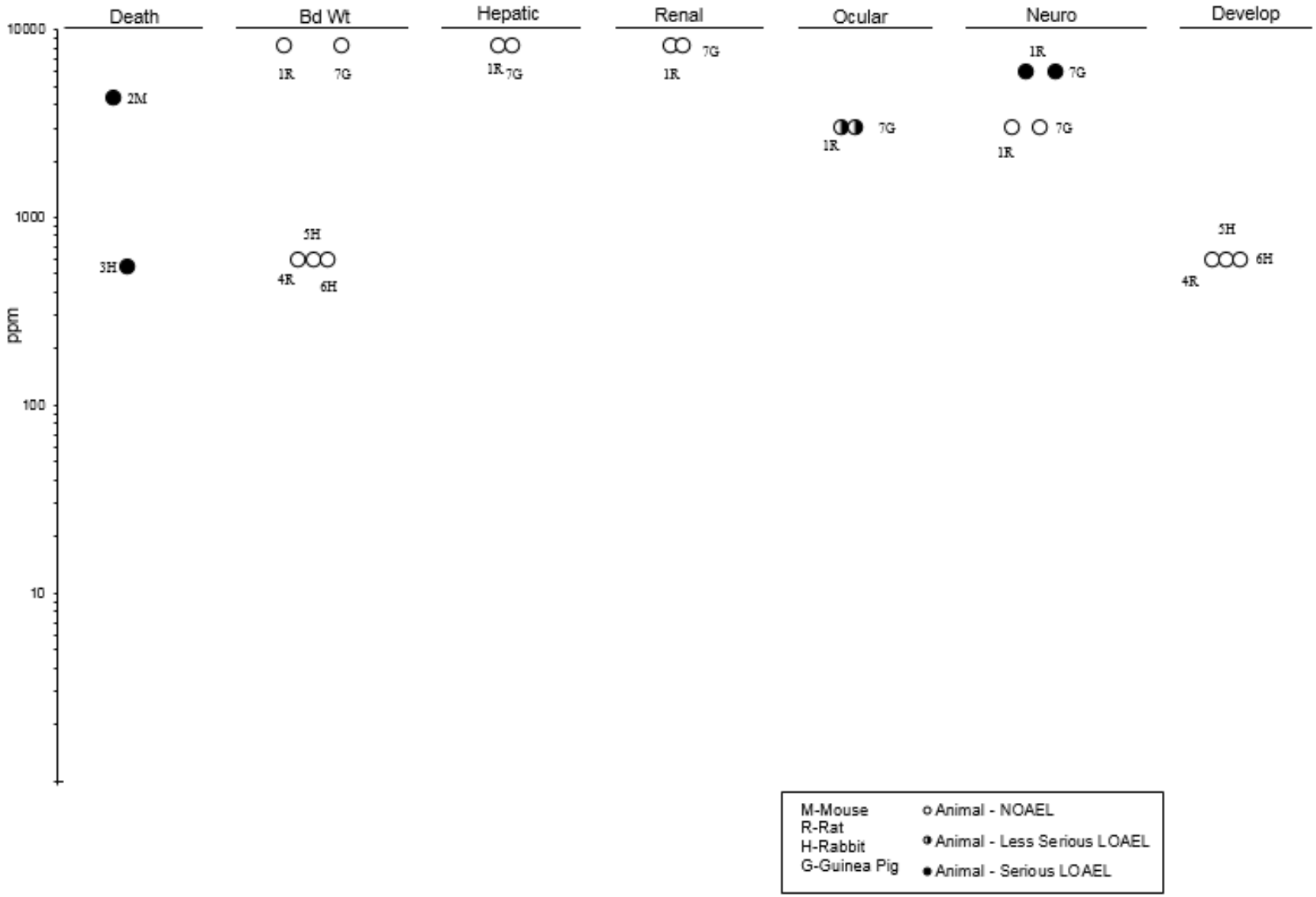


Figure 2-2. Levels of Significant Exposure to Chlorobenzene – Inhalation

Intermediate (15-364 days)

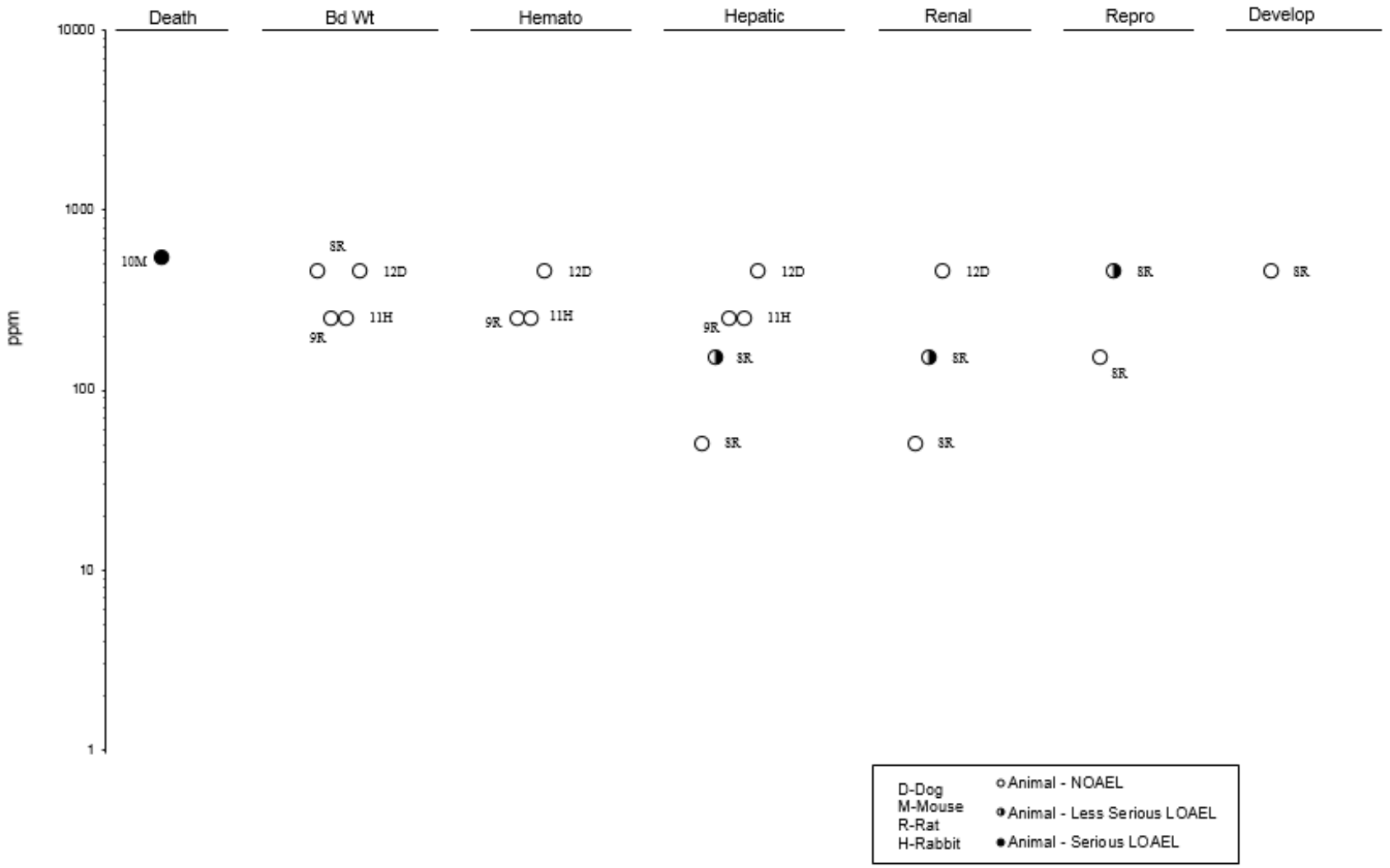


Table 2-2. Levels of Significant Exposure to Chlorobenzene – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
ACUTE EXPOSURE									
1	Rat (F344/N) 5 M, 5 F	Once (GO)	250, 500, 1,000, 2,000, 4,000	CS, LE	Death			4,000	3/5 males and 4/5 females died
					Neuro	2,000		4,000	Prostration
NTP 1985 (data also reported in Kluwe et al. 1985)									
2	Rat (albino) 22 F	GDs 6–15 1 time/day (GO)	0, 100, 300	BW, CS, DX, FX, LE, MX, TG	Bd wt	300			
					Develop	300			
Monsanto Co. 1977									
3	Rat (F344/N) 5 M, 5 F	14 days 1 time/day (GO)	0, 125, 250, 500, 1,000, 2,000	BW, CS, GN, LE	Death			1,000	5/5 males and 5/5 females died
					Bd wt	500			
					Neuro	500		1,000	Prostration
NTP 1985 (data also reported in Kluwe et al. 1985)									
4	Mouse (B6C3F1) 5 M, 5 F	Once (GO)	250, 500, 1,000, 2,000, 4,000	CS, LE	Death			1,000 M 2,000 F	5/5 males died 5/5 females died
NTP 1985 (data also reported in Kluwe et al. 1985)									
5	Mouse (B6C3F1) 5 M, 5 F	14 days 1 time/day (GO)	0, 30, 60, 125, 250, 500	BW, CS, GN, LE	Bd wt	500			
NTP 1985 (data also reported in Kluwe et al. 1985)									

Table 2-2. Levels of Significant Exposure to Chlorobenzene – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
INTERMEDIATE EXPOSURE									
6	Rat (F344/N) 10 M, 10 F	13 weeks 5 days/week 1 time/day (GO)	0, 60, 125, 250, 500, 750	BC, BW, CS, GN, HE, HP, LE, OW, UR	Death			500	4/10 males and 3/10 females died
					Bd wt	125 M 250 F	250 M 500 F		12% lower mean final body weight
					Hepatic	125 M 60 F	250 M 125 F	750 M, F	Males: 24% increased mean relative liver weight at 250 mg/kg/day; hepatic degeneration/necrosis at 750 mg/kg/day Females: 19% increased mean relative liver weight at 125 mg/kg/day; hepatic degeneration/necrosis at 750 mg/kg/day
					Renal	250	500	750 F	13–15% increased mean relative kidney weight at 500 mg/kg/day; renal necrosis/degeneration in females at 750 mg/kg/day
					Immuno	500	750		Myeloid depletion in bone marrow, lymphoid depletion in spleen
NTP 1985 (data also reported in Kluwe et al. 1985)									
7	Rat (albino) 18 M, 18 F	3 months 1 time/day (GO)	Controls (untreated), 12.5, 50, 100, 250	BC, BW, CS, FI, GN, HE, HP, LE, OF, OW, UR	Bd wt	250			
					Hemato	250			
					Hepatic	100	250		27–29% increased liver weight
					Renal	100	250		13–14% increased kidney weight
Monsanto Co. 1967b									

Table 2-2. Levels of Significant Exposure to Chlorobenzene – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
8	Mouse (B6C3F1) 10 M, 10 F	13 weeks 5 days/week 1 time/day (GO)	0, 60, 125, 250, 500, 750	BC, BW, CS, GN, HE, HP, LE, OW, UR	Death			250	5/9 males and 4/10 females died
					Bd wt	125 M 250 F	250 M 500 F		15–20% lower mean final body weight at lethal dose levels
					Hepatic	60 M 125 F	125 M	250 M, F	At 125 mg/kg/day: 14% increased mean relative liver weight in males At 250 mg/kg/day: 29–35% increased mean relative liver weight and hepatic necrosis/degeneration
					Renal	125		250	Renal necrosis/degeneration
					Immuno	125		250	Males: lymphoid depletion/necrosis in thymus and spleen; myeloid depletion in bone marrow Females: lymphoid depletion/necrosis in spleen
NTP 1985 (data also reported in Kluwe et al. 1985)									
9	Dog (beagle) 4 M, 4 F	13 weeks 5 days/week 1 time/day (C)	0, 28, 55, 280	BC, BW, CS, FI, GN, HE, HP, LE, OF, OW, UR	Death			280	2/4 dogs of each sex died
					Bd wt	55		280	Emaciation, weight loss at lethal dose
					Hemato	55	280		Males: decreased hematocrit, hemoglobin, RBCs; increased lymphocytes Females: decreased hemoglobin, RBCs, total WBCs

Table 2-2. Levels of Significant Exposure to Chlorobenzene – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Hepatic	28[b] M 55 F	55 M	280	At 55 mg/kg/day: 22% increased liver weight in males; bile duct hyperplasia (2/4) males At 280 mg/kg/day: increased liver weight (56% in males, 77% in females); degenerative liver lesions in most males and females ($BMDL_{10}$ of 9.59 mg/kg)
					Renal	55		280	Increased kidney weight (62% in males, 87% in females); degenerative kidney lesions in most males and females
Monsanto Co. 1967a									
CHRONIC EXPOSURE									
10	Rat (F344/N) 50 M, 50 F	103 weeks 5 days/week 1 time/day (GO)	0, 60, 120	BW, CS, GN, HP, LE	Death			120	Decreased survival
					Bd wt	120			
					Hepatic	120			
					Renal	120			
					Cancer			120	CEL: neoplastic liver nodules
NTP 1985 (data also reported in Kluwe et al. 1985)									

Table 2-2. Levels of Significant Exposure to Chlorobenzene – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
11	Mouse (B6C3F1) 50 M, 50 F	103 weeks 5 days/week 1 time/day (GO)	Males: 0, 30, 60 Females: 0, 60,120	BW, CS, GN, HP, LE	Bd wt	60 M 120 F			
					Hepatic	60 M 120 F			
					Renal	60 M 120 F			

NTP 1985 (data also reported in Kluwe et al. 1985)

[a]The number corresponds to entries in Figure 2-3; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-3. Where such differences exist, only the levels of effect for the most sensitive gender are presented.
[b]Used to derive an intermediate-duration oral MRL of 0.07 mg/kg/day; based on a $BMDL_{10}$ of 9.59 mg/kg, adjusted to continuous duration exposure ($BMDL_{ADJ}$ of 6.85 mg/kg/day) and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

BC = serum (blood) chemistry; Bd wt or BW = body weight; (C) = capsule; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; F = female(s); FI = food intake; FX = fetal toxicity; (GO) = gavage in oil; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MX = maternal toxicity; Neuro = neurological; NOAEL = no-observed-adverse-effect level; OF = organ function; OW = organ weight; RBC = red blood cell; TG = teratogenicity; UR = urinalysis; WBC = white blood cell

Figure 2-3. Levels of Significant Exposure to Chlorobenzene – Oral

Acute (≤14 days)

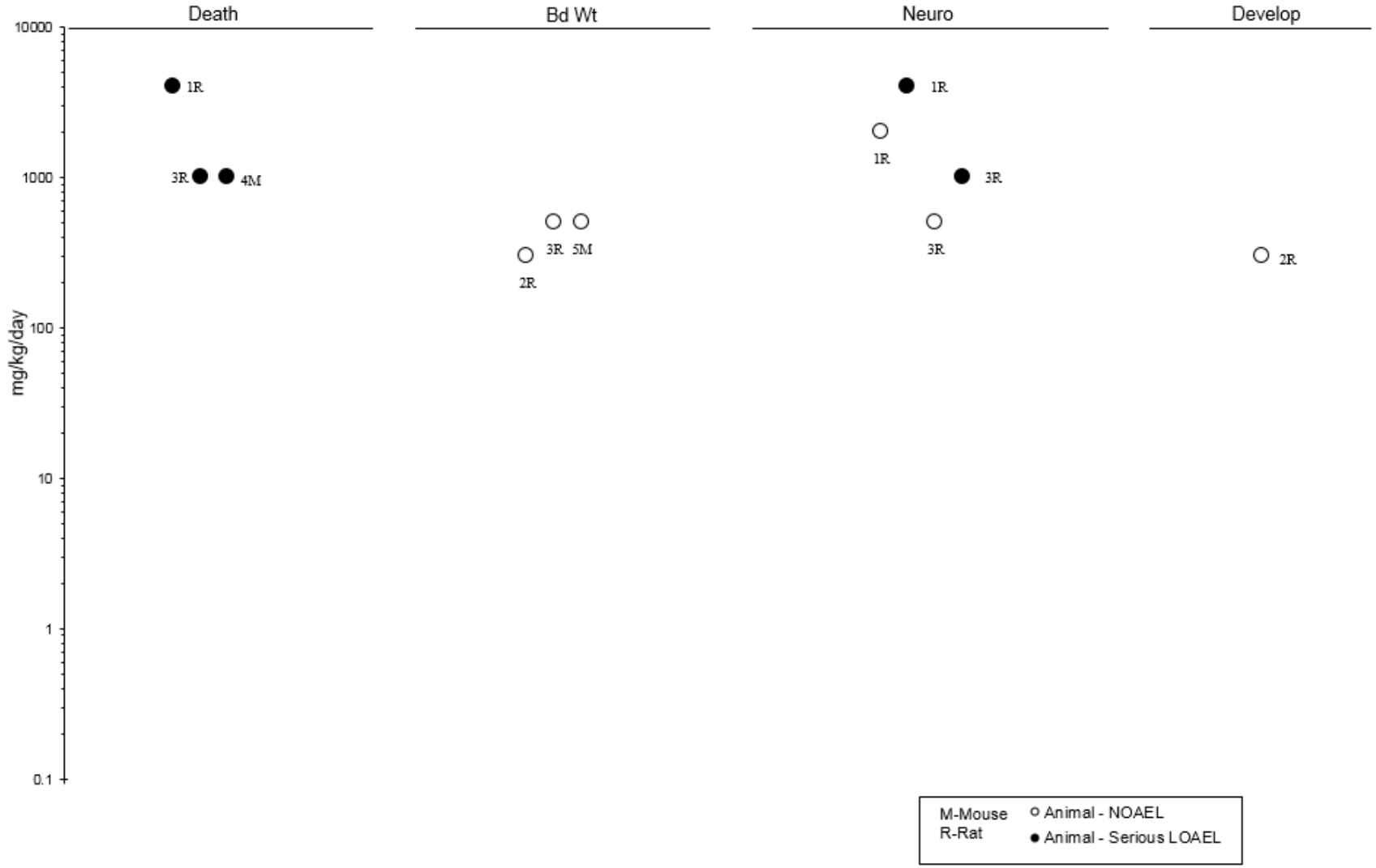


Figure 2-3. Levels of Significant Exposure to Chlorobenzene – Oral

Intermediate (15-364 days)

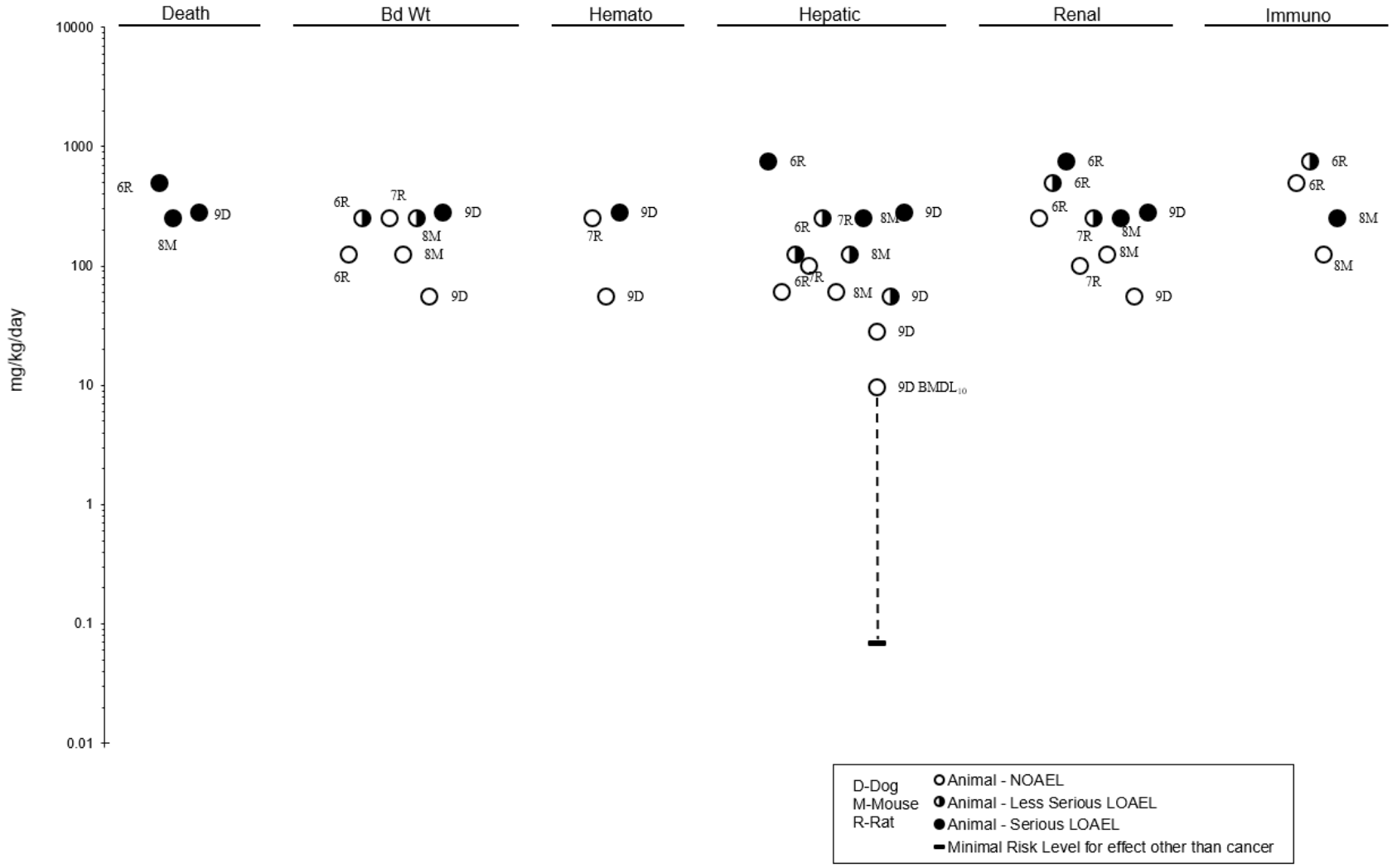
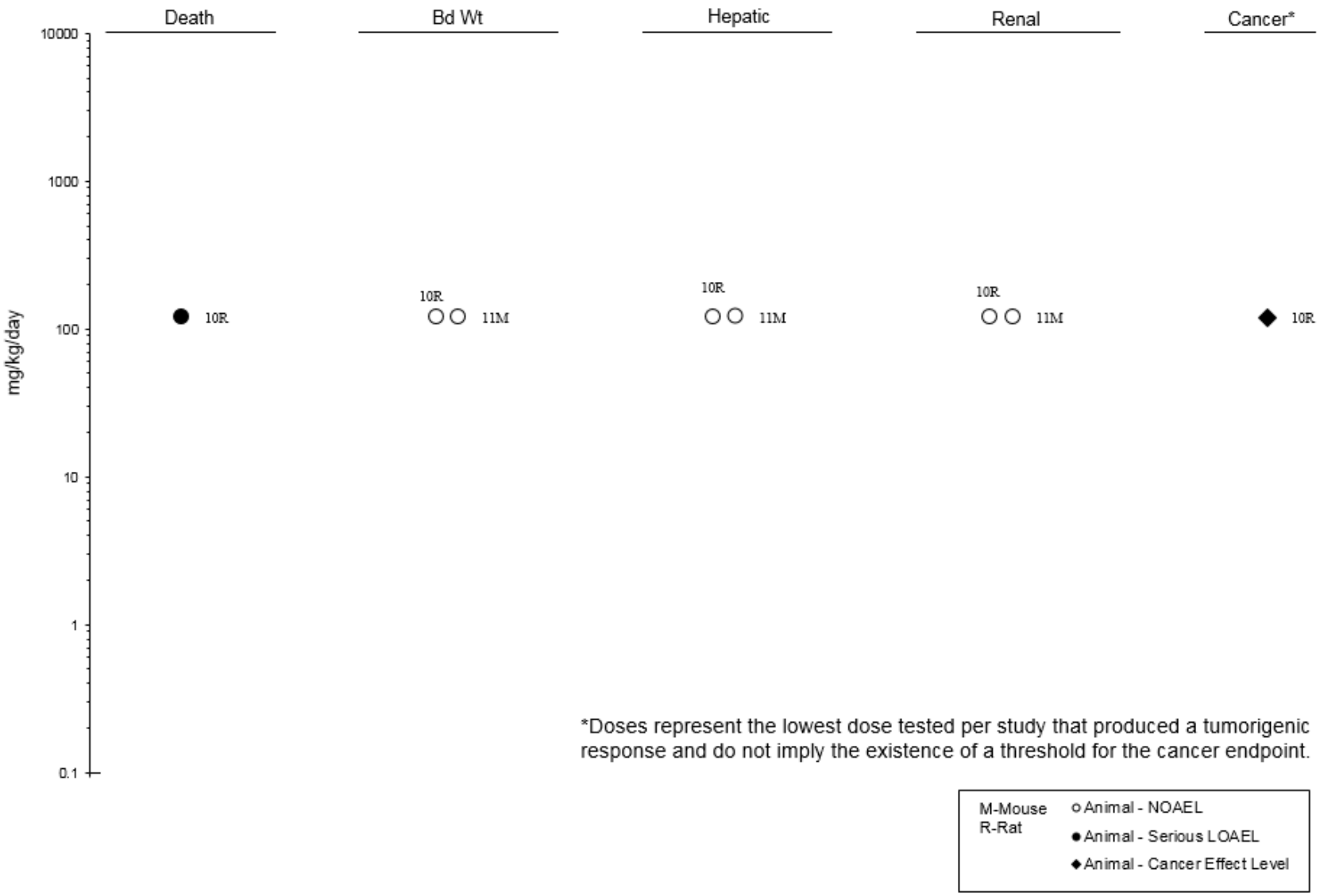


Figure 2-3. Levels of Significant Exposure to Chlorobenzene – Oral

Chronic (≥365 days)



2.2 DEATH

There have been no documented human deaths from chlorobenzene exposure.

The acute lethality of chlorobenzene is relatively low in animals. Single exposure of mice to chlorobenzene vapor at 4,300 ppm for 2 hours resulted in 100% mortality (Rozenbaum et al. 1947). Rabbits died 2 weeks after a 2-hour inhalation exposure at approximately 537 ppm (Rozenbaum et al. 1947). In a 3-week study of mice exposed to chlorobenzene vapor for 7 hours/day at 543 ppm, death was reported in 5/10 mice (Zub 1978). Death occurred in 3/5 male and 4/5 female rats within 2–3 days following a single gavage dose at 4,000 mg/kg; similar exposure of mice resulted in 100% mortality of males at 1,000 mg/kg and females at 2,000 mg/kg (NTP 1985). In a 14-day repeated-dose study of rats, gavage exposure at doses ≥1,000 mg/kg resulted in 100% lethality (NTP 1985). In 13-week repeated-dose studies, survival was reduced in male and female rats gavaged at doses ≥500 and male and female mice gavaged at doses ≥250 mg/kg/day (NTP 1985). In a 13-week oral study of dogs, ingestion of chlorobenzene at 280 mg/kg/day resulted in death of 2/4 dogs of each sex (Monsanto Co. 1967a). In a 2-year oral rat study, survival of males at 120 mg/kg/day was significantly lower than that of vehicle controls (NTP 1985).

2.3 BODY WEIGHT

No exposure-related effects on body weight were observed in laboratory animals repeatedly exposed to chlorobenzene vapor at concentrations as high as 250–590 ppm (NIOSH 1977; John et al. 1984; Monsanto Co. 1980; Nair et al. 1987). In a 13-week gavage study, chlorobenzene exposure of male and female rats and mice at 250 mg/kg/day (males) and 500 mg/kg/day (females) resulted in 12–20% depressed mean final body weight (NTP 1985). Dogs, which were exposed for 13 weeks to chlorobenzene by daily capsule, exhibited emaciation and weight loss at a lethal dose of 280 mg/kg/day (Monsanto Co. 1967a).

2.4 RESPIRATORY

Available information regarding chlorobenzene-induced respiratory effects is limited to observations of nose rubbing behavior among guinea pigs exposed to chlorobenzene vapor for 30 minutes at a concentration as low as 2,990 ppm (Shell Oil Co. 1991).

Chlorobenzene has been used as a model VOC in several *in vitro* studies to investigate possible mechanisms of lung inflammation (Feltens et al. 2010; Fischäder et al. 2008; Lehmann et al. 2008; Röder-Stolinski et al. 2008).

2.5 CARDIOVASCULAR

No studies were located regarding cardiovascular effects in humans or laboratory animals exposed to chlorobenzene.

2.6 GASTROINTESTINAL

No studies were located regarding gastrointestinal effects in humans or laboratory animals exposed to chlorobenzene.

2.7 HEMATOLOGICAL

Information regarding the potential for inhaled chlorobenzene to cause hematological effects is limited. In studies of rats and rabbits exposed to chlorobenzene vapor for 7 hours/day, 5 days/week, for up to 24 weeks at concentrations of 75 or 250 ppm, NIOSH (1977) reported exposure concentration-related effects on RBC parameters (primarily an increase in reticulocyte count). Other hematological parameters (RBC count, hemoglobins, hematocrit, and WBC count) were variable and were comparable to controls at the end of the test. Zub (1978) reported slight leukopenia and lymphocytosis in mice exposed to chlorobenzene for 7 hours/day for 3 months at 21.7 ppm, and similar effects in mice similarly exposed for up to 3 weeks at 543 ppm (Zub 1978). However, limited details in the study report and lack of supportive evidence from other animal studies preclude meaningful evaluation of chlorobenzene-induced hematological effects following inhalation exposure. Monsanto Co. (1967a) reported changes in selected blood parameters in dogs receiving chlorobenzene in daily capsule at 280 mg/kg/day for 13 weeks. High-dose males exhibited decreases in hematocrit, hemoglobin, and RBCs, and increased lymphocytes; high-dose females exhibited decreased in hemoglobin, RBCs, and total WBCs.

2.8 MUSCULOSKELETAL

No studies were located regarding musculoskeletal effects in humans or laboratory animals exposed to chlorobenzene.

2.9 HEPATIC

Available information regarding the potential for chlorobenzene to cause adverse liver effects in humans is limited to a single case report in which ingestion of 140 mL of 90% chlorobenzene by a suicidal 40-year-old, 58-kg, male with a daily alcohol intake of 200 g resulted in severe liver necrosis (Babany et al. 1991; Reygagne et al. 1992). Although daily alcohol consumption was estimated at approximately 200 g, the patient had no history of chronic liver disease. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels on the third day after chlorobenzene ingestion were 345 and 201 times, respectively, the upper limit of normal. Liver biopsy revealed centrilobular and mediolobular necrosis.

Results from animal studies identify the liver as a target of chlorobenzene toxicity. In a 2-generation inhalation study of rats, repeated inhalation exposure to chlorobenzene vapor at 150 ppm resulted in statistically significantly increased mean relative liver weight and increased incidence of hepatocellular hypertrophy of parental males (incidence data not included in the study report) (Nair et al. 1987). In several studies that employed repeated gavage exposure to chlorobenzene for up to 3 months, increased liver weight was reported in rats at doses as low as 100–250 mg/kg/day (Monsanto Co. 1967b; NTP 1985), mice at 125 mg/kg/day (NTP 1985), and male dogs at 55 mg/kg/day (Monsanto Co. 1967a). Two of four male dogs treated at 55 mg/kg/day exhibited bile duct hyperplasia (a lesion not observed in control dogs); bile duct hyperplasia was noted in 4/4 male and 3/4 female dogs dosed at 280 mg/kg/day (Monsanto Co. 1967a). Hepatic degeneration/necrosis were observed in rats treated at 750 mg/kg/day and mice treated at 250 mg/kg/day (NTP 1985). There were no apparent exposure-related hepatic effects among rats or mice administered chlorobenzene by gavage for up to 2 years at doses as high as 60–120 mg/kg/day (NTP 1985).

2.10 RENAL

Available animal data implicate the kidney as a target of chlorobenzene toxicity. Nair et al. (1987) reported tubular dilatation with eosinophilic material, interstitial nephritis, and foci of regenerative epithelium in 2 generations of parental male rats exposed to chlorobenzene vapor at concentrations ≥150 ppm. In 3-month repeated-dose gavage studies of rats and mice (NTP 1985), increased kidney weight was observed at doses ≥500 mg/kg/day. Histopathologic kidney lesions (degeneration/focal necrosis of the proximal tubules) were observed in rats at 750 mg/kg/day and in mice at doses ≥250 mg/kg/day (NTP 1985). Kidney lesions (e.g., tubule dilatation, epithelial degeneration, vacuolation) were observed in dogs treated with chlorobenzene in capsules for 13 weeks at 280 mg/kg/day (Monsanto

Co. 1967a). There were no indications of exposure-related kidney effects in rats or mice administered chlorobenzene by gavage for up to 2 years at doses as high as 60–120 mg/kg/day (NTP 1985).

2.11 DERMAL

Limited information was located regarding chlorobenzene-induced dermal effects. There were no signs of dermal sensitization in a guinea pig dermal sensitization assay in which chlorobenzene was applied via intradermal injection (induction at 1% chlorobenzene), followed by topical induction of a 50% solution and two challenge dermal applications of a 25% solution (Miles Inc. 1984).

2.12 OCULAR

Limited information was located regarding chlorobenzene-induced ocular effects. Lacrimation and squinting behavior was observed among rats and guinea pigs exposed to chlorobenzene vapor for 30 minutes at concentrations ≥2,990 ppm (Shell Oil Co. 1991). Mild to moderate corneal opacity, iritis, redness, chemosis, and discharge were among the effects observed in the eyes of rabbits following ocular instillation of chlorobenzene (Zeneca Specialties 1982).

2.13 ENDOCRINE

No studies were located regarding endocrine effects in humans or laboratory animals exposed to chlorobenzene.

2.14 IMMUNOLOGICAL

Histological studies in mice and rats suggest that chlorobenzene has immunotoxic properties. In a 13-week oral study of rats, gavage exposure at 750 mg/kg/day resulted in myeloid depletion in bone marrow and lymphoid depletion in the spleen (NTP 1985). Similar exposure of mice at doses ≥250 mg/kg/day resulted in lymphoid depletion/necrosis in the thymus and spleen and myeloid depletion in bone marrow of males and lymphoid depletion/necrosis in the spleen of females (NTP 1985). Since no human data were located regarding immunotoxic effects and no animal studies that evaluated immune function are available, firm conclusions cannot be made concerning the potential for chlorobenzene to affect the immune system in humans.

2.15 NEUROLOGICAL

Chlorobenzene affects the central nervous system. Humans occupationally exposed to chlorobenzene intermittently for up to 2 years displayed signs of neurotoxicity including numbness, cyanosis (from depression of respiratory center), hyperesthesia, and muscle spasms (Rozenbaum et al. 1947). Specific exposure levels and histopathologic data were not provided in the study report. When four volunteers were exposed via inhalation to 60.2 ppm chlorobenzene for 7 hours during a study of urinary metabolites in exposed workers, all complained of disagreeable odor and drowsiness, three complained of headache, two of throbbing pain in eyes, and one of sore throat (Ogata et al. 1991). A test of critical flicker fusion frequency was also conducted. The critical flicker fusion rate is the frequency at which an intermittent light stimulus is perceived as continuous; the test is used to evaluate the rate of perceptual temporal processing capacity. The mean flicker-fusion value declined 3.1 cycles/second in exposed subjects, compared to controls (Ogata et al. 1991).

Neurological effects of chlorobenzene have also been reported in animals following inhalation. Acute inhalation exposure produced muscle spasms followed by narcosis in rabbits exposed to 1,090 ppm chlorobenzene for >2 hours (Rozenbaum et al. 1947). Ataxia and narcosis were observed in rats exposed to chlorobenzene vapor for 30 minutes at concentrations ≥5,850 ppm; most rats displayed these effects within 25 minutes following initiation of exposure, but they recovered rapidly after removal from the test chamber (Shell Oil Co. 1991). At an exposure concentration of 7,970 ppm, tremors were observed as well. In addition to the narcotic effects observed in the rats, similarly-exposed guinea pigs also exhibited salivation at 7,970 ppm.

There is a paucity of data on the effects of chlorobenzene in humans following oral exposure. A 2-year-old male swallowed 5–10 cc of a stain remover, which consisted almost entirely of chlorobenzene. He became unconscious, did not respond to skin stimuli, showed muscle spasms, and became cyanotic. The odor of chlorobenzene could be detected in his urine and exhaled air; however, the child recovered uneventfully (Reich 1934).

Available information regarding the potential for chlorobenzene to cause neurological effects following oral exposure in laboratory animals is limited to findings of decreased activity and prostration among rats administered chlorobenzene by gavage once at 4,000 mg/kg/day or repeatedly for 14 days at 1,000 mg/kg/day; these doses were also lethal (NTP 1985).

2.16 REPRODUCTIVE

No studies were located regarding reproductive effects in humans exposed to chlorobenzene.

Limited information is available regarding the potential for chlorobenzene-induced reproductive effects in laboratory animals. In a two-generation study of rats intermittently exposed to chlorobenzene vapor from at least 10 weeks prior to mating through lactation of their progeny, increased incidences of degenerative testicular changes were observed in males of both generations (6/30 versus 1/30 among controls; p=0.051) exposed at 450 ppm (Nair et al. 1987). The toxicological significance of this finding is unclear because mean mating, pregnancy, and male fertility indices for both F0 and F1 generations were comparable for all groups.

2.17 DEVELOPMENTAL

No studies were located regarding developmental effects in humans exposed to chlorobenzene.

Limited information is available regarding the potential for chlorobenzene-induced developmental effects in laboratory animals. No indications of chlorobenzene exposure-related developmental effects were observed in studies of rats and rabbits exposed to chlorobenzene vapor for 6 hours/day at concentrations as high as 590 ppm during gestation days 6–15 (rats) or 6–18 (rabbits) (John et al. 1984). No indications of exposure-related developmental effects were observed in a study of rats administered chlorobenzene by daily gavage at doses up to 300 mg/kg/day during gestation days 6–15 (Monsanto Co. 1977).

2.18 OTHER NONCANCER

No studies were located regarding other noncancer effects.

2.19 CANCER

In a chronic oral bioassay in rats and mice (NTP 1985), there was no evidence for carcinogenicity in either sex of mice or in female rats administered chlorobenzene in corn oil by gavage at dose levels up to 120 mg/kg/day. Increased tumor frequencies were not seen in female rats or in male or female mice. Male rats showed a significant ($p<0.05$) increase in the incidence of neoplastic nodules of the liver in the 120 mg/kg/day dose group, but no increases were found at lower doses. While progression from nodules to carcinomas is a well-characterized phenomenon, existing data are inadequate to characterize the

carcinogenic potential of chlorobenzene in humans. EPA (IRIS 2003) assigned chlorobenzene to class D (not classifiable as to human carcinogenicity), based on lack of human data, inadequate animal data, and predominantly negative genetic toxicity data in bacterial, yeast, and mouse lymphoma cells.

2.20 GENOTOXICITY

No studies were located regarding the genotoxic effects of chlorobenzene in humans. The potential genotoxicity of chlorobenzene has been evaluated in several *in vivo* studies (Table 2-3) and a greater number of *in vitro* assays (Table 2-4). Collectively, the results indicate that chlorobenzene is not likely to act as a mutagen; however, *in vivo* results indicate that chlorobenzene may induce other genotoxic effects. However, as shown in Figure 3-1, chlorobenzene undergoes CYP450 catalyzed oxidation to form the 3,4- and 2,3-epoxides of chlorobenzene. Both epoxides can be formed in liver and lung (and other tissues such as kidney and adrenal cortex) and are capable of covalently binding to deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and proteins.

Table 2-3. Genotoxicity of Chlorobenzene *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Drosophila:			
Male germ cells (airborne exposure)	Sex-linked recessive lethal mutations	–	Bioassay Systems Corp. 1982
Mammalian cells:			
Rat bone marrow (intraperitoneal injection)	Chromosomal aberrations	+	Siddiqui et al. 2006
Mouse bone marrow (intraperitoneal injection)	Chromosomal aberrations	+	Mohtashumipur et al. 1987
Rat bone marrow (intraperitoneal injection)	Micronuclei	+	Siddiqui et al. 2006
Mouse bone marrow (intraperitoneal injection)	Micronuclei	+	Mohtashumipur et al. 1987
Mouse bone marrow (intraperitoneal injection)	Micronuclei	–	Shelby and Witt 1995
Mouse bone marrow (intraperitoneal injection)	Micronuclei	–	Shelby et al. 1993
Mouse peripheral lymphocytes (intraperitoneal injection)	DNA damage	+	Vaghef and Hellman 1995
Mouse bone marrow (intraperitoneal injection)	DNA damage	–	Vaghef and Hellman 1995

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

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

Species (test system)	Endpoint	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Gene mutation	–	–	E.I. DuPont 1977
S. typhimurium TA98, TA100, TA1535, TA1537	Gene mutation	–	–	Haworth et al. 1983; NTP 1985
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Gene mutation	–	–	Monsanto Co. 1976a, 1976b
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Gene mutation	–	–	Shimizu et al. 1983
Saccharomyces cerevisiae D4	Gene mutation	–	–	Monsanto Co. 1976a, 1976b
Eukaryotic organisms:				
Aspergillus nidulans	Gene mutation	–	No data	Prasad 1970
Mammalian cells:				
Mouse L5178Y lymphoma cells	Gene mutation	+	+/–	McGregor et al. 1988
Mouse L5178Y lymphoma cells	Gene mutation	–	–	Monsanto Co. 1976c
Rat liver epithelial cells	Cell transformation	No data	+	Shimada et al. 1983
Rat hepatocytes	DNA repair	No data	–	Shimada et al. 1983
Chinese hamster ovary cells	Chromosomal aberrations	–	–	Bioassay Systems Corp. 1982
Chinese hamster ovary cells	Chromosomal aberrations	–	–	Loveday et al. 1989
Chinese hamster ovary cells	Sister chromatid exchange	–	+	Loveday et al. 1989

+ = positive result; – = negative result; +/– = inconclusive results; DNA = deoxyribonucleic acid

Chlorobenzene did not induce sex-linked recessive lethal mutations in male *Drosophila* germ cells (Bioassay Systems Corp. 1982). Chlorobenzene induced chromosomal aberrations and micronuclei in bone marrow cells in two assays that employed intraperitoneal injection of chlorobenzene into rats and mice (Mohtashumipur et al. 1987; Siddiqui et al. 2006), but did not induce micronuclei in mouse bone marrow cells in other similarly-designed studies (Shelby and Witt 1995; Shelby et al. 1993). Vaghef and Hellman (1995) reported DNA damage in peripheral lymphocytes from mice following intraperitoneal

injection of chlorobenzene for 3 days at 750 mg/kg/day, but no evidence of DNA damage to bone marrow cells.

Chlorobenzene did not induce gene mutations either in the presence or absence of exogenous metabolic activation in bacterial assays that employed multiple strains of *Salmonella typhimurium* (E.I. DuPont 1977; Haworth et al. 1983 [also reported in NTP 1985]; Monsanto Co. 1976a, 1976c; Shimizu et al. 1983) or the D4 strain of *Saccharomyces cerevisiae* (Monsanto Co. 1976a, 1976b). Chlorobenzene did not induce gene mutations in the fungus *Aspergillus nidulans* in the presence of exogenous metabolic activation (Prasad 1970). Positive results for chlorobenzene-induced gene mutations in mouse L5178Y lymphoma cells in the presence and absence of exogenous metabolic activation were obtained in one study (McGregor et al. 1988), but not in another study (Monsanto Co. 1976c). Chlorobenzene induced cell transformation in rat liver epithelial cells, but did not induce DNA repair in rat hepatocytes (Shimada et al. 1983). In Chinese hamster ovary cells, negative results were obtained for chromosomal aberrations in the presence and absence of exogenous metabolic activation (Bioassay Systems Corp. 1982; Loveday et al. 1989), but positive results were obtained for sister chromatic exchange in the absence of exogenous metabolic activation (Loveday et al. 1989).