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 chlorophenols. 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.

This Toxicological Profile addresses the health effects of the 13 chlorophenols listed below.

Compound	Abbreviation	CAS Registry Number
2-Chlorophenol	2-CP	95-57-8
4-Chlorophenol	4-CP	106-48-9
2,3-Dichlorophenol	2,3-DCP	576-24-9
2,4-Dichlorophenol	2,4-DCP	120-83-2
2,5-Dichlorophenol	2,5-DCP	583-78-8
3,4-Dichlorophenol	3,4-DCP	95-77-2
3,5-Dichlorophenol	3,5-DCP	591-35-5
2,3,4-Trichlorophenol	2,3,4-TCP	15950-66-0
2,4,5-Trichlorophenol	2,4,5-TCP	95-95-4
2,4,6-Trichlorophenol	2,4,6-TCP	88-06-2
2,3,4,5-Tetrachlorophenol	2,3,4,5-TeCP	4901-51-3
2,3,4,6-Tetrachlorophenol	2,3,4,6-TeCP	58-90-2
2,3,5,6-Tetrachlorophenol	2,3,5,6-TeCP	935-95-5

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. Figures 2-1 (2-CP), 2-2 (4-CP), 2-3 (2,4-DCP), 2-4, (2,4,5-TCP), 2-5 (2,4,6-CP), 2-6 (2,3,4,6-TeCP), and 2-7 (other chlorophenols) 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 chlorophenols, but may not be inclusive of the entire body of literature.

Animal inhalation studies are presented in Table 2-1 and Figure 2-8. Animal oral studies are presented in Table 2-2 and Figure 2-9 (2-CP), Table 2-3 and Figure 2-10 (4-CP), Table 2-4 and Figure 2-11 (2,4-DCP), Table 2-5 and Figure 2-12 (2,4,5-TCP), Table 2-6 and Figure 2-13 (2,4,6-TCP), Table 2-7 and Figure 2-14 (2,3,4,6-TeCP), and Table 2-8 and Figure 2-15 (other chlorophenols). Animal dermal studies are presented in Table 2-9.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects (SLOAELs) are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is 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 2,4,6-TCP are indicated in Table 2-6 and Figure 2-13.

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.

The discussion of the available data for health effects in this chapter begins with an overview of the health effects and comparisons across the different chlorophenols (except pentachlorophenol, which is addressed in a separate toxicological profile). Human studies, which are generally not specific to an individual chlorophenol, are discussed next. Finally, compound-specific subsections describe the animal data; these subsections are provided in order from monochlorophenols through di-, tri-, and tetrachlorophenols (in the order shown in the list above). If there are no data for a given chlorophenol, there is no subsection for that compound. Apart from acute lethality data in animals, no information was located on the health effects of the following chlorophenols in humans or animals exposed by any route: 2,3-DCP, 3,4-DCP, and 2,3,4-TCP. Toxicity data on 2,3,4,5- and 2,4,5,6-TeCP were limited to acute oral lethality and acute dermal toxicity studies.

A total of 56 human studies of chlorophenols were identified in the literature searches. Only three of these, case reports of dermal exposure to 2,4-DCP, are included in the study counts in the figures. The remaining studies of humans exposed to chlorophenols largely fell into two categories: (1) studies of workers exposed to mixtures of chlorophenols, chlorophenoxy compounds and other herbicides, and, often, tetrachlorodibenzo-*p*-dioxin (TCDD); or (2) studies that use urinary chlorophenol concentrations in the general population, often from the National Health and Nutrition Examination Survey (NHANES), as a measure of exposure. Because of the co-exposures in studies of occupationally-exposed persons, it is typically not possible to attribute any observed effects to chlorophenols either as a group or individually. While studies using urinary concentrations to assess exposure often provide data on individual chlorophenols, the presence of these compounds in urine does not conclusively indicate exposure to chlorophenols, as they may occur in urine as metabolites of other compounds, including chlorobenzenes (Billi et al. 1985; Yoshida et al. 2002), hexachlorocyclohexanes (Engst et al. 1976; Koransky et al. 1975), lindane (Karapally et al. 1973), VC-13 (Shafik et al. 1973), 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (Hill et al. 1989), or higher chlorophenols (Renner and Mucke 1986). Further discussion of this issue is provided in Section 3.3.1 (Biomarkers of Exposure). Although the human studies of occupational exposure and studies that use urinary chlorophenol levels to assess exposure are not included in the study counts, these studies are discussed in this chapter as they provide some (albeit limited) information that is useful for hazard identification.

The results of human and animal studies suggest that the chlorophenols discussed in this profile induce effects on the liver, central nervous system, body weight, and reproductive function. In addition, kidney effects have been observed after exposure to 2,4,5-TCP and 2,3,4,6-TeCP, while 2,4-DCP has shown immune system effects.

- **Hepatic effects:** No human data are available. In animals exposed orally, hepatic effects including clinical chemistry changes, increased liver weight, hepatocellular hypertrophy, and necrosis have been observed in rats and mice exposed to 2-CP, 4-CP, 2,4-DCP, 2,4,5-TCP, 2,4,6-TCP, and 2,3,4,6-TeCP.

- **Reproductive effects:** No reliable human data are available. Animal studies of oral exposure have shown decreases in implantations, litter size, and/or live births per litter after exposure to 4-CP, 2,4-DCP, and 2,4,6-TCP. Adverse effects on the male reproductive system (increases in abnormal sperm and decreases in sperm motility) were seen in mice after oral exposure to 2,4-DCP.

- **Neurological effects:** A case report of human fatality after 2,4-DCP exposure reported that the victim had seizures prior to death. In animals exposed orally to 2- and 4-CP and 2,4-DCP or via dermal exposure to tetrachlorophenols, clinical signs of neurotoxicity including lethargy, tremors, convulsions, and/or central nervous system depression were observed. There were no human or animal studies examining sensitive measures of neurotoxicity.

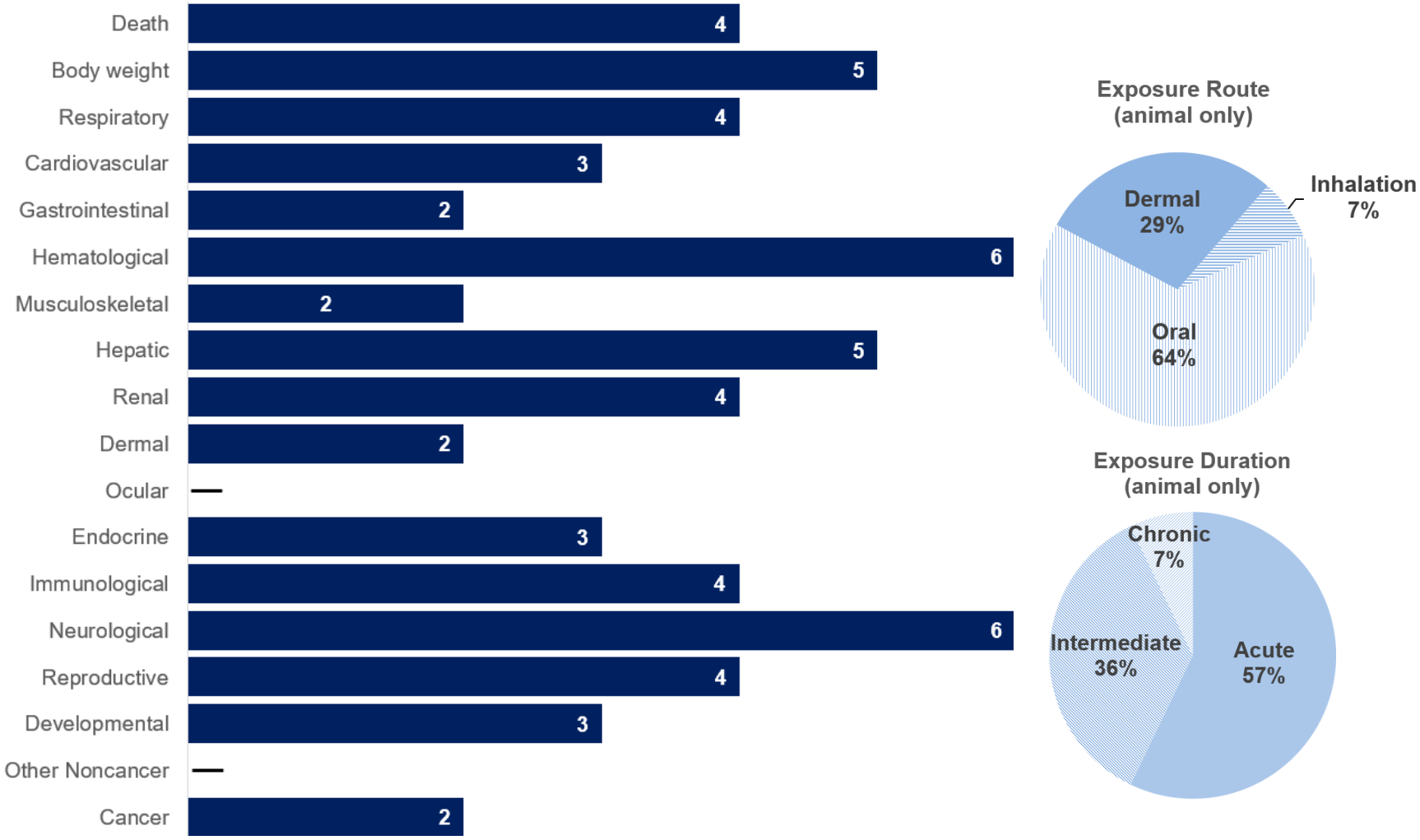
- **Body weight effects:** No human data are available. Body weight decreases or reductions in body weight gain were noted after acute-, intermediate-, and/or chronic-duration oral exposures to 2-CP, 4-CP, 2,4-DCP, 2,4,5-TCP, 2,4,6-TCP, and 2,3,4,6-TeCP.

- **Immunological effects:** No human data are available. The limited data from studies examining sensitive measures of immune system function show that 2,4-DCP decreases delayed-type hypersensitivity and increases antibody production, but 2-CP and 2,4,6-TCP did not induce similar effects.

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

Most studies examined the potential hematological, neurological, body weight, and hepatic effects of 2-chlorophenol

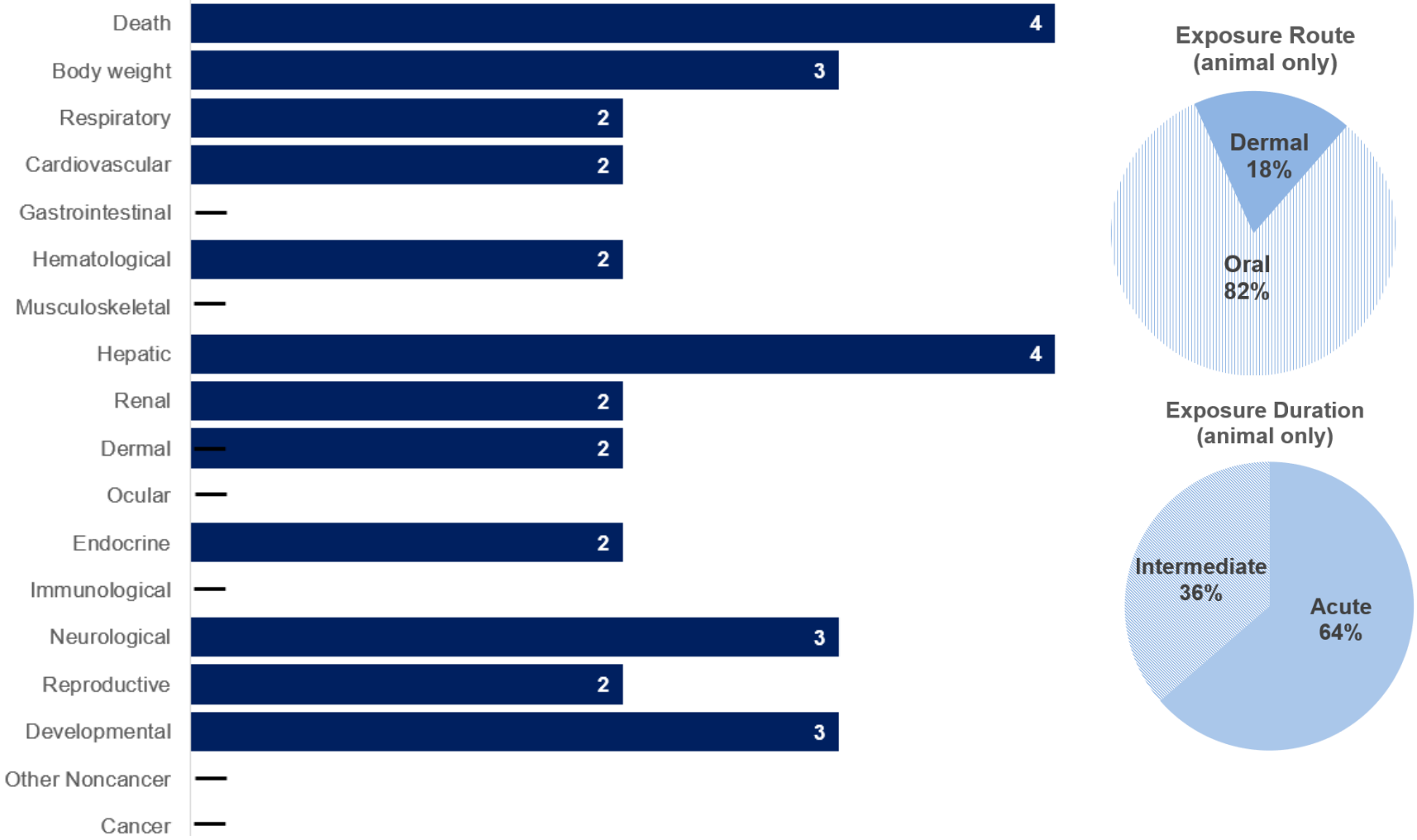
All studies pertaining specifically to 2-chlorophenol are in animals (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 14 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

Figure 2-2. Overview of the Number of Studies Examining 4-Chlorophenol Health Effects*

Most studies examined the potential mortality and hepatic effects of 4-chlorophenol
All studies pertaining specifically to 4-chlorophenol are in animals (counts represent studies examining endpoint)

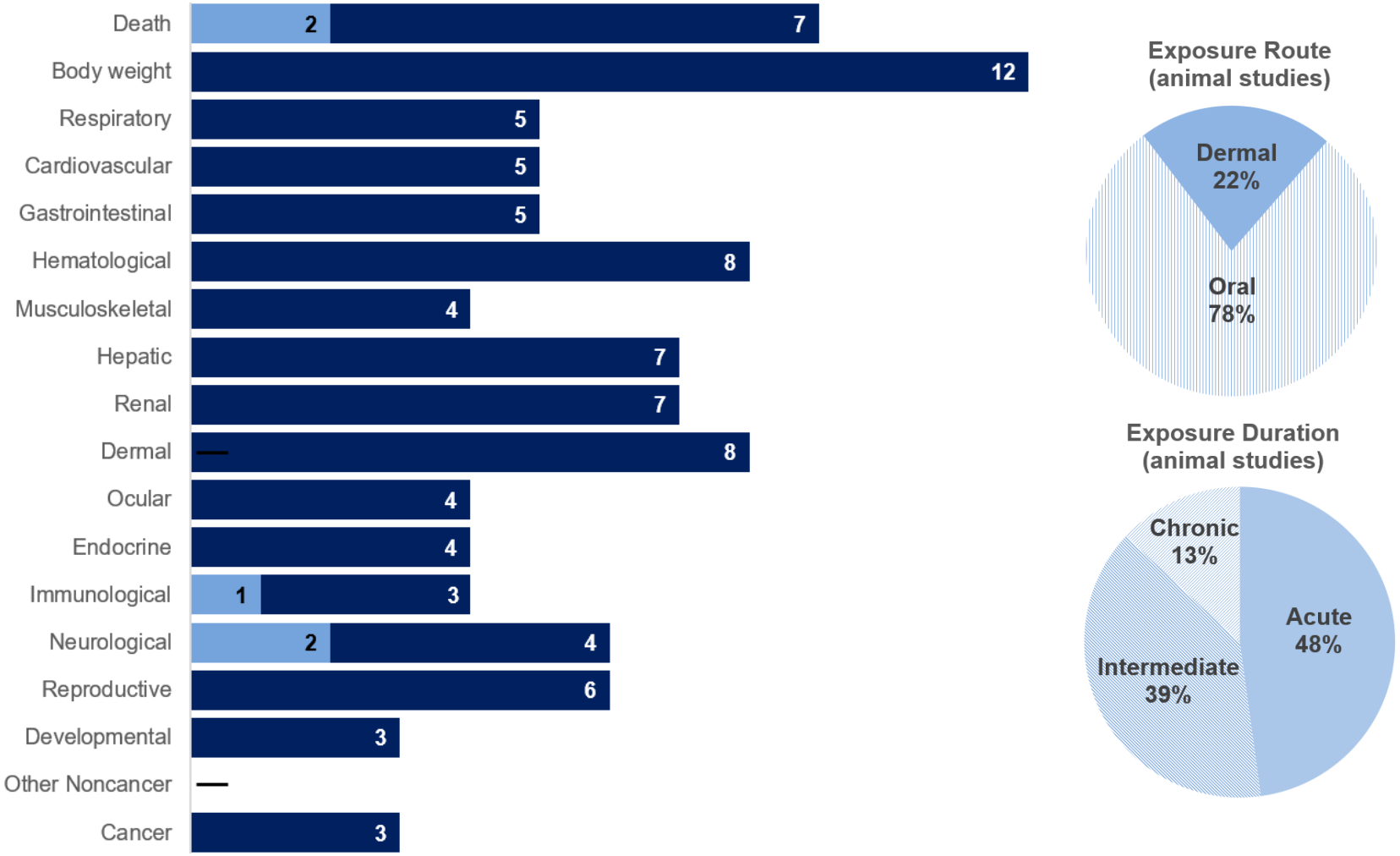


*Includes studies discussed in Chapter 2. A total of 11 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

Figure 2-3. Overview of the Number of Studies Examining 2,4-Dichlorophenol Health Effects*

Most studies examined the potential mortality, body weight, hematological, and dermal effects of 2,4-dichlorophenol

Only 3 **human** studies examined 2,4-dichlorophenol health effects; the rest are in **animals** (counts represent studies examining endpoint)

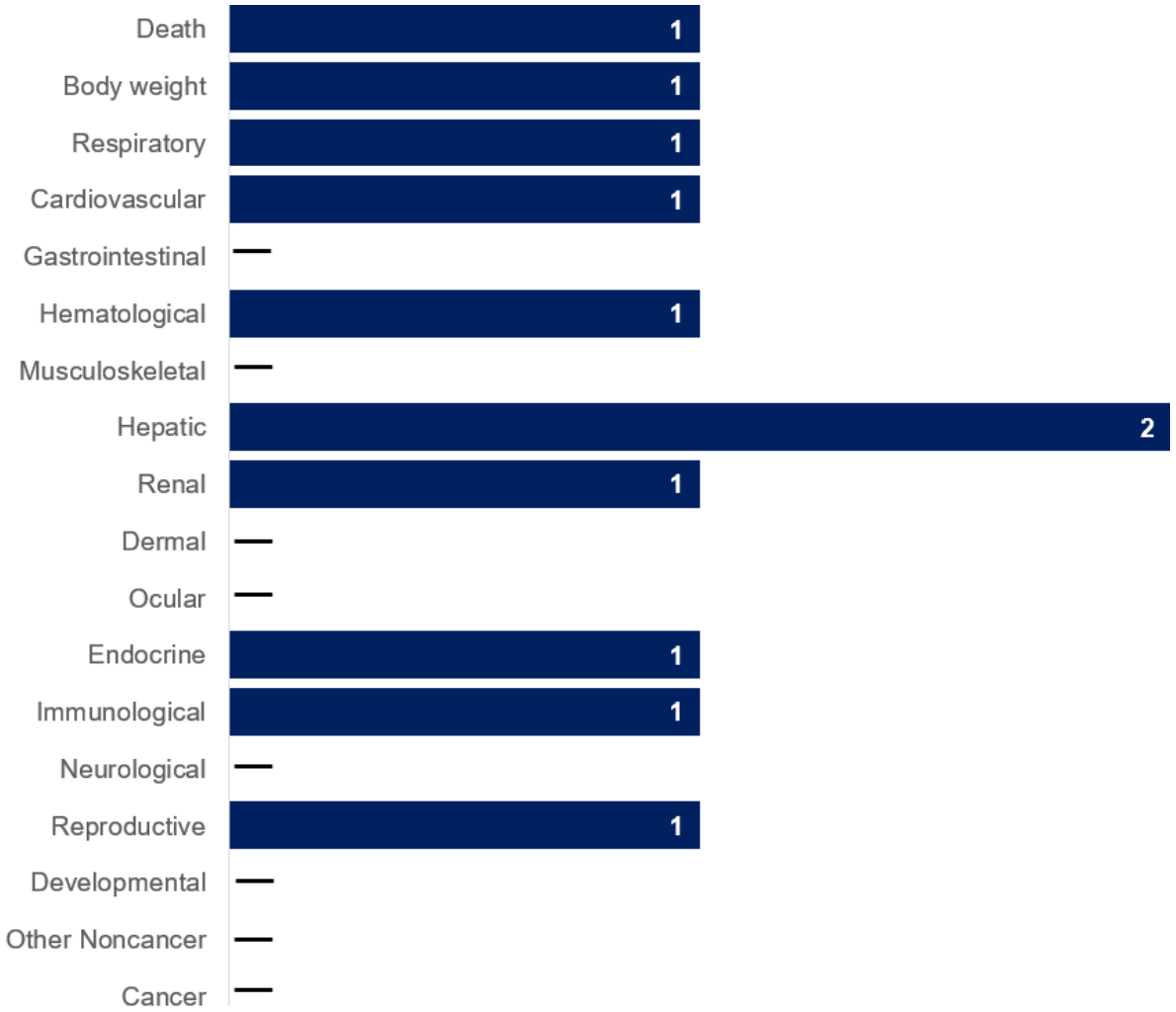


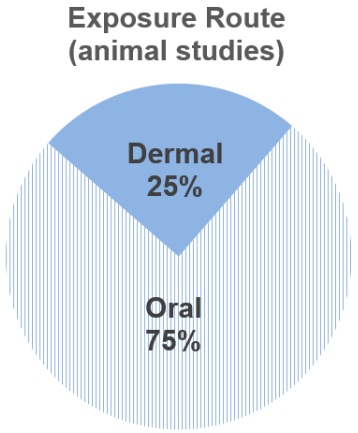
*Includes studies discussed in Chapter 2. A total of 26 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

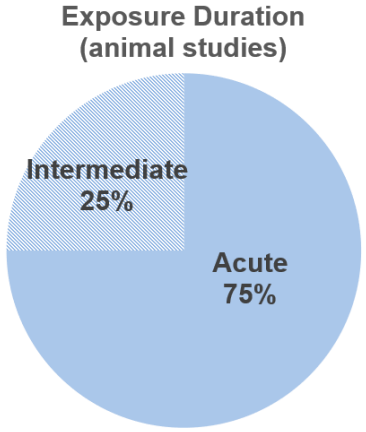
Figure 2-4. Overview of the Number of Studies Examining 2,4,5-Trichlorophenol Health Effects*

Most studies examined the potential hepatic effects of 2,4,5-trichlorophenol

All studies pertaining specifically to 2,4,5-trichlorophenol are in **animals** (counts represent studies examining endpoint)



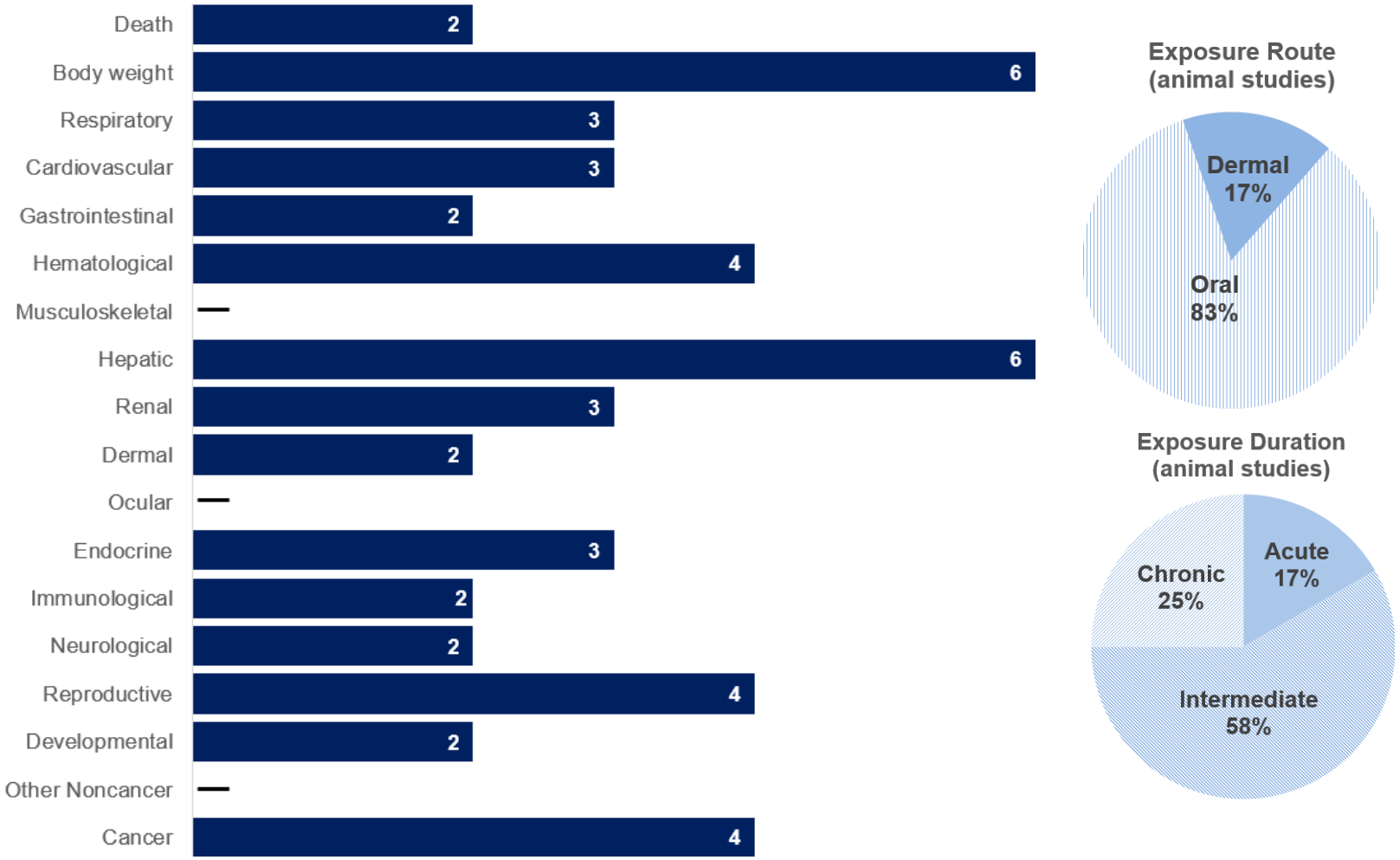




*Includes studies discussed in Chapter 2. A total of 4 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

Figure 2-5. Overview of the Number of Studies Examining 2,4,6-Trichlorophenol Health Effects*

Most studies examined the potential body weight and hepatic effects of 2,4,6-trichlorophenol
All studies pertaining specifically to 2,4,6-trichlorophenol are in **animals** (counts represent studies examining endpoint)

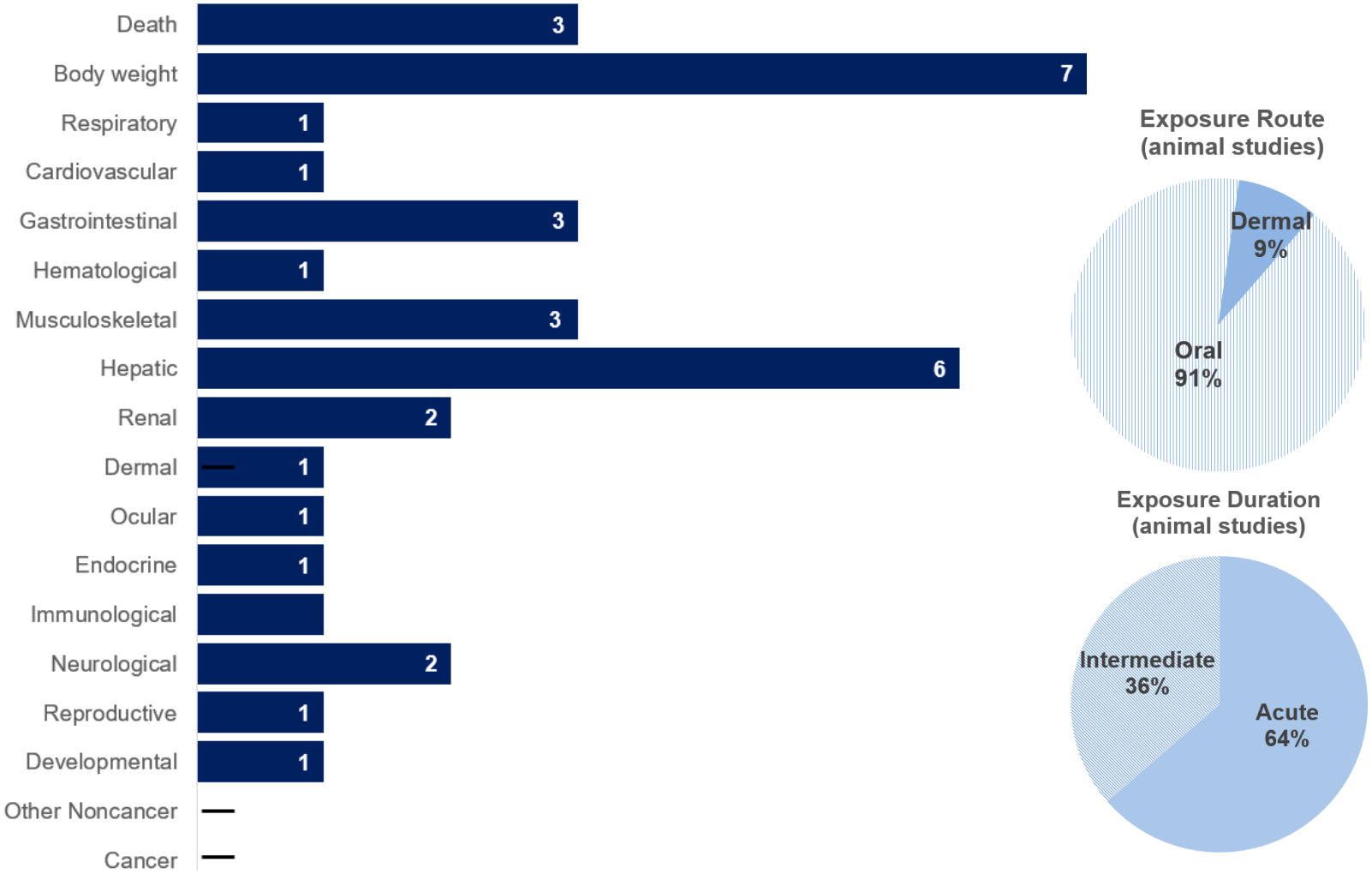


*Includes studies discussed in Chapter 2. A total of 12 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

Figure 2-6. Overview of the Number of Studies Examining 2,3,4,6-Tetrachlorophenol Health Effects*

Most studies examined the potential body weight and hepatic effects of 2,3,4,6-tetrachlorophenols

All studies pertaining specifically to 2,3,4,6-tetrachlorophenol are in animals (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 11 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

Figure 2-7. Overview of the Number of Studies Examining Other Chlorophenol Health Effects*

All studies examined the potential mortality, neurological, and dermal effects of other chlorophenols

All studies pertaining specifically to other chlorophenols are in **animals** (counts represent studies examining endpoint)

*Includes studies discussed in Chapter 2. A total of 10 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects
ACUTE EXPOSURE									
1	Rat (Wistar) 5 M, 5 F	4 hours	17, 104, 908	BW, GN, CS, LE	Bd wt	908			
					Resp	104	908 M		Tachypnea in 1/5 rats
					Neuro	104	908		Restlessness, hunched posture
2-Chlorophenol Rhone-Poulenc 1991									

[a]The number corresponds to entries in Figure 2-8; differences in levels of health effects and cancer effects between male and females are not indicated in the figure. Where such differences exist, only the levels of effect for the most sensitive sex are presented.

Bd wt or BW = body weight; CS = clinical signs; F = female(s); GN = gross necropsy; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Neuro = neurotoxicity; NOAEL = no-observed-adverse-effect level; Resp = respiratory

Figure 2-8. Levels of Significant Exposure to Chlorophenols – Inhalation

Acute (≤14 days)

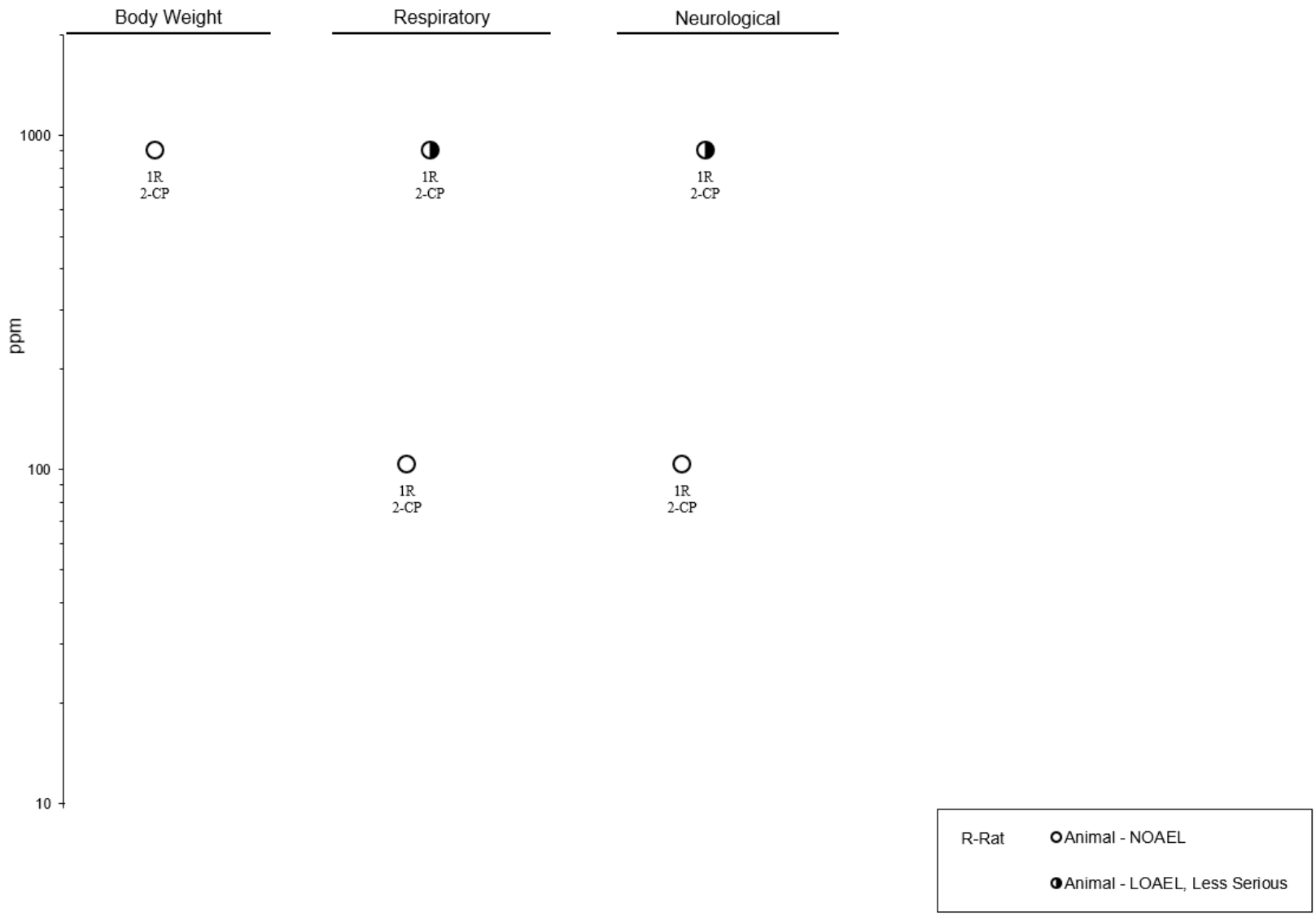


Table 2-2. Levels of Significant Exposure to 2-Chlorophenol – Oral

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)	Effects
ACUTE EXPOSURE									
1	Rat (Sprague-Dawley) 10	10 days (GO)	0, 13, 64, 129, 257	CS, BW, FI, WI, BC, HE, OW, GN, HP	Bd wt	257			
					Resp	257			
					Cardio	257			
					Gastro	257			
					Hemato	257			
					Musc/skel	257			
					Hepatic	257			
					Renal	257			
					Endocr	257			
					Immuno	257			
					Neuro	257			No effect on brain weight or brain or sciatic nerve histology
					Repro	257			No effect on gonad weights or reproductive organ histology
Daniel et al. 1993									
2	Rat (Sprague-Dawley) 12	9 days; PNDs 4–12 (GO)	0, 20, 100, 500	LE	Death			500	All rats died by 9th day of dosing in dose range-finding study
Hasegawa et al. 2005									
3	Mouse (CD-1 ICR) 12 M, 12 F	14 days (GO)	0, 35, 69, 175	BW, OW, GN, BC, CS, BI, LE, OF, HE	Death			175	24/24 died
					Bd wt	35	69		Decreased body weight
					Hemato	69			
					Hepatic	69			
					Renal	69			
					Immuno	69			
					Neuro		35		Hyperactivity
Borzelleca et al. 1985a									

Table 2-2. Levels of Significant Exposure to 2-Chlorophenol – 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)	Effects
4	Mouse (CD-1 ICR) 10 M, 10 F	Once (GW)	NS	CS, LE	Death			345 F	LD_{50}
Borzelleca et al. 1985a, 1985b									
INTERMEDIATE EXPOSURE									
5	Rat (Sprague-Dawley)	Dams: from weaning through mating at PND 90, gestation, and lactation Offspring: from conception through weaning (PND 21) and for additional 12 weeks (W)	0, 0.76, 7.6, 76	BW, DX, RX, OF, OW, HP	Bd wt	76			
					Hemato	76			
					Hepatic	76			
					Immuno	76			
					Repro	7.6[b]	76		Decreased mean litter size and increased percent stillborn
					Develop	76			No effect on weaning weight or survival to weaning
Exon and Koller 1982, 1983a, 1983b, 1985									
6	Rat (Sprague-Dawley) 10	90 days (GO)	0, 17, 50, 150	CS, BW, FI, WI, BC, HE, OW, GN, HP	Bd wt	150			
					Resp	150			
					Cardio	150			
					Gastro	150			
					Hemato	150			
					Musc/skel	150			
					Hepatic	150			
					Renal	150			
					Endocr	150			
					Immuno	150			

Table 2-2. Levels of Significant Exposure to 2-Chlorophenol – 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)	Effects
					Neuro	150			No effect on brain weight or brain or sciatic nerve histology
					Repro	150			No effect on gonad weights or reproductive organ histology
Daniel et al. 1993									
7	Rat (Sprague-Dawley) 12/sex	28 days (GO)	0, 8, 40, 200, 1,000	BW, CS, HE, BI, GN, OW, HP, DX	Bd wt	1,000			
					Resp	1,000			
					Cardio	1,000			
					Hemato	1,000			
					Hepatic	200	1,000		Increased incidence slight centrilobular hepatocellular hypertrophy
					Renal	1,000			
					Endocr	1,000			
					Neuro	500		1,000	Increased incidence of tremors (9/24), hypoactivity (13/24), and abnormal gait (11/24)
					Repro	1,000			No effect on histopathology of testes, epididymides, ovaries, or uteri
					Develop	1,000			
Hasegawa et al. 2005									
8	Rat (Sprague-Dawley) 12/sex	PNDs 4–21 (GO)	0, 8, 50, 300	BW, CS, HE, BI, GN, OW, HP, DX	Neuro	50		300	Increased incidence of tremors (23/24 combined, compared with 0/24 control)
					Develop	50	300		Increased incidences basophilic renal tubules
Hasegawa et al. 2005									

Table 2-2. Levels of Significant Exposure to 2-Chlorophenol – 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)	Effects
CHRONIC EXPOSURE									
9	Rat (Sprague-Dawley) 24–32 M, F	From conception through weaning (PND 21) and until death or 24 months (W)	0, 0.62, 6.2, 62	HE, HP	Hemato	62			
Exon and Koller 1985									

[a]The number corresponds to entries in Figure 2-9; differences in levels of health effects and cancer effects between male and females are not indicated in the figure. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an intermediate-duration MRL of 0.08 mg/kg/day. The NOAEL of 7.6 mg/kg/day was divided by a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). See Appendix A for details.

BC = blood chemistry; Bd wt or BW = body weight; BI = biochemical changes; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental effects; Endocr = endocrine; F = female(s); FI = food intake; FX = fetal toxicity; (GO) = gavage in oil; (GW) = gavage in water; Gastro = gastrointestinal; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; LD_{50} = dose producing 50% death; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; Musc/skel = muscular skeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function; OW = organ weight; PND = postnatal day; Repro = reproductive; Resp = respiratory; RX = reproductive effects; (W) = water; WI = water intake

Figure 2-9. Levels of Significant Exposure to 2-Chlorophenol – Oral
Acute (≤14 days)

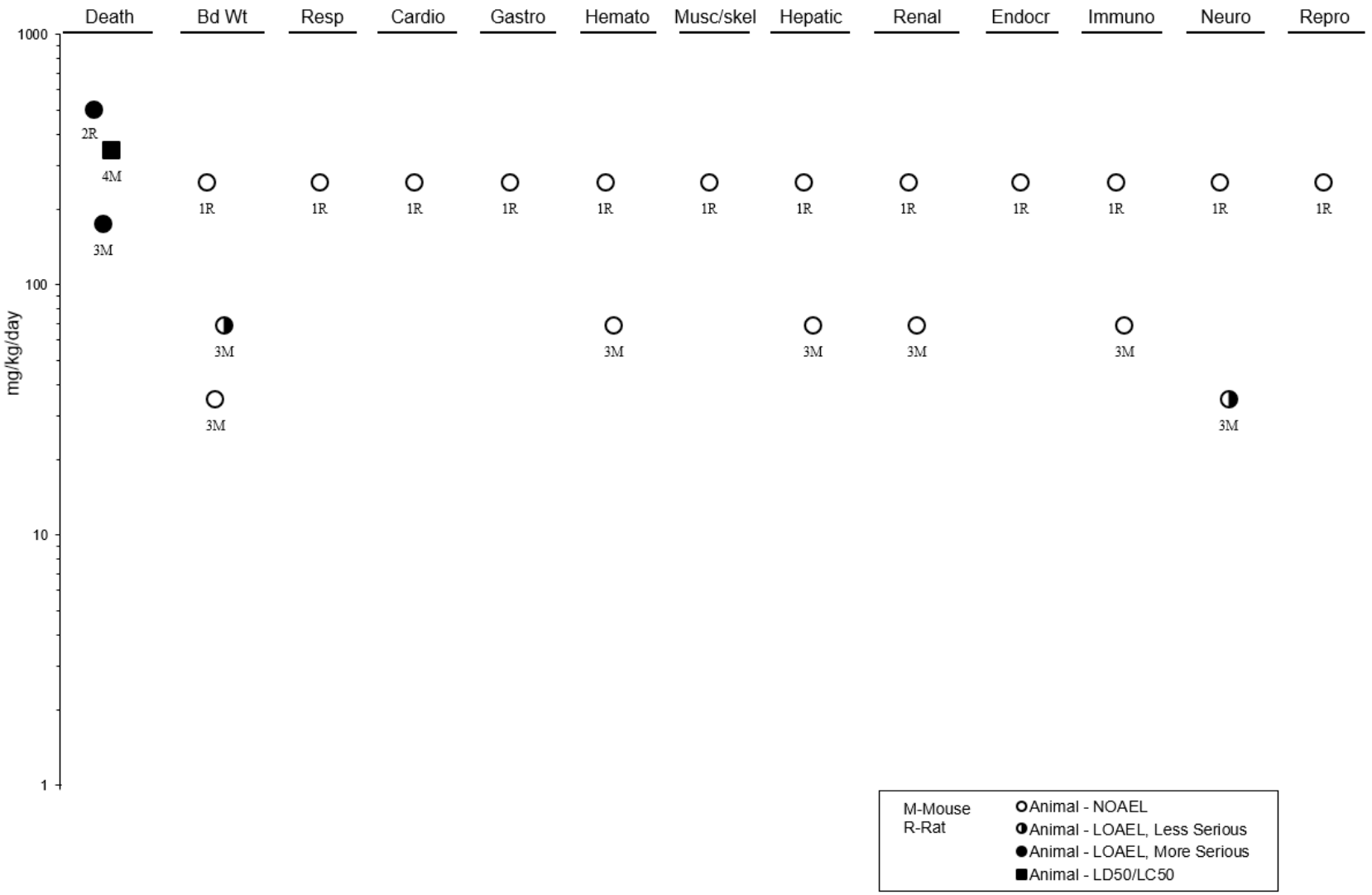


Figure 2-9. Levels of Significant Exposure to 2-Chlorophenol – Oral
Intermediate (15-364 days)

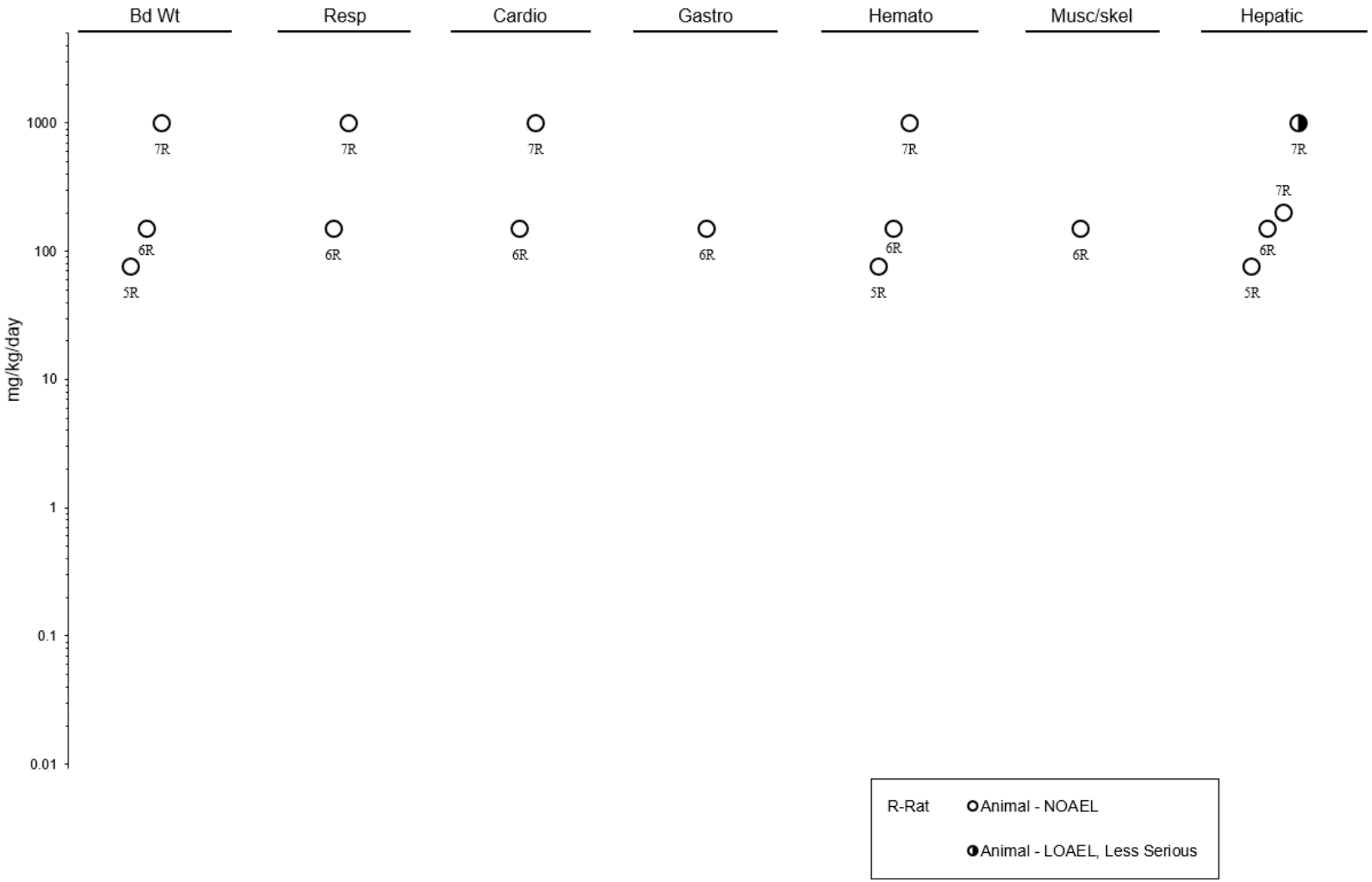


Figure 2-9. Levels of Significant Exposure to 2-Chlorophenol – Oral

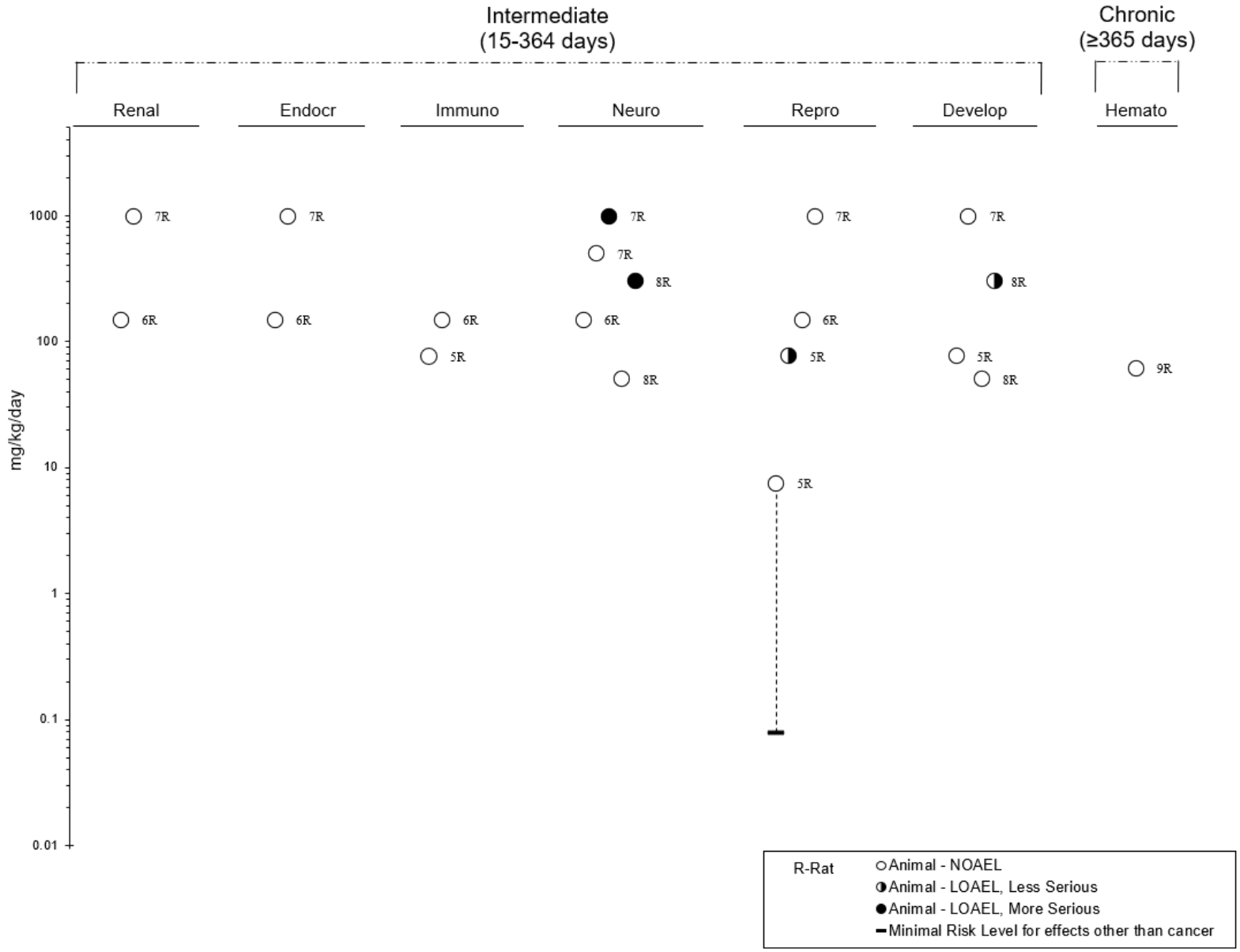


Table 2-3. Levels of Significant Exposure to 4-Chlorophenol – Oral

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)	Effects
ACUTE EXPOSURE									
1	Rat (Sprague-Dawley) 6–13 F	Once on GD 11 (G)	0, 100, 333, 667, 1,000	BW, MX, DX	Bd wt	667 F	1,000 F		Maternal body weight loss of 10 g in 24 hours
					Develop	1,000			
Kavlock 1990									
2	Rat (Sprague-Dawley) 4–9 M	2 weeks 7 days/week (GO)	0, 0.32, 0.64, 1.28, 2.58, 5.2, 10.2, 20.6	OW, BC, BI	Hepatic	2.58 M			Foamy cytoplasm and clustering of mitochondria and endoplasmic reticulum in hepatocytes was not considered adverse
Phornchirasilp et al. 1989b									
3	Rat (Sprague-Dawley) 12	12 days 7 days/week (GO)	0, 40, 200, 1,000	CS, BW, FI, FX, DX, GN, OW, HP	Death			1,000	6/12 males and 5/12 females died in first 12 days dosing
					Neuro	200		1,000	Tremors, clonic convulsions
BSRC 2011									
4	Mouse (CD-1 ICR) 10 M,10 F	Once (GO)	NS	CS, LE	Death			1,373 M	LD_{50}
Borzelleca et al. 1985a, 1985b									
5	Mouse (ICR) 10 F	Once (GO)	0, 700, 1,050, 1,575	CS, LE, OW	Death			1,050 F	1/10 mice died
Shi et al. 2013									
INTERMEDIATE EXPOSURE									
6	Rat (Sprague-Dawley) 4–6 M	4–8 weeks 7 days/week (GO)	0, 0.64, 1.28, 5.2	HP, BC	Hepatic	0.64 M			Foamy cytoplasm and clustering of mitochondria and endoplasmic reticulum in hepatocytes not considered adverse
Phornchirasilp et al. 1989b									

Table 2-3. Levels of Significant Exposure to 4-Chlorophenol – 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)	Effects
7	Rat (Sprague-Dawley) 12/sex	28 days 7 days/week (GO)	0, 20, 100, 500	BW, CS, HE, BI, GN, OW, HP	Bd wt	500			
					Neuro	100		500	Increased incidence of tremors (23/24), rapid breathing (20/24), and salivation (17/24)
					Repro	500			No effect on histopathology of testes, epididymides, ovaries, or uteri
Hasegawa et al. 2005									
8	Rat (Sprague-Dawley) 12/sex	PNDs 4–21 (GO)	0, 12, 60, 300, 500	BW, CS, HE, BI, GN, OW, HP, DX	Death			500	4/4 males and 3/4 females died in dose range-finding study
					Resp	300			
					Cardio	300			
					Hemato	300			
					Hepatic	300			
					Renal	300			
					Endocr	300			
					Neuro	60		300	Increased incidence of tremors (24/24)
					Develop	300			
Hasegawa et al. 2005									

Table 2-3. Levels of Significant Exposure to 4-Chlorophenol – 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)	Effects
9	Rat (Sprague-Dawley) 12	41–53 days 7 days/week (GO)	0, 40, 200, 1,000	CS, BW, FI, FX, DX, GN, OW, HP	Repro	40[b]	200		Significantly reduced number live births ($BMDL_{1SD}$ =85.77 mg/kg/day); reduced number implantation sites
					Develop	200			
BSRC 2011									

[a]The number corresponds to entries in Figure 2-10; differences in levels of health effects and cancer effects between male and females are not indicated in the figure. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an intermediate-duration oral MRL of 0.9 mg/kg/day using BMD analysis. The $BMDL_{1SD}$ of 85.77 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). See Appendix A for details.

BC = blood chemistry; Bd wt or BW = body weight; BI = biochemical changes; BMD = benchmark dose; BMDL = benchmark dose, lower confidence limit; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental effects; Endocr = endocrine; F = female(s); FI = food intake; FX = fetal toxicity; (G) = gavage; (GO) = gavage in oil; GD = gestation day; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; LD_{50} = dose producing 50% death; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; MX = maternal toxicity; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OW = organ weight; PND = postnatal day; Repro = reproductive; Resp = respiratory; SD = standard deviation

Figure 2-10. Levels of Significant Exposure to 4-Chlorophenol – Oral
Acute (≤14 days)

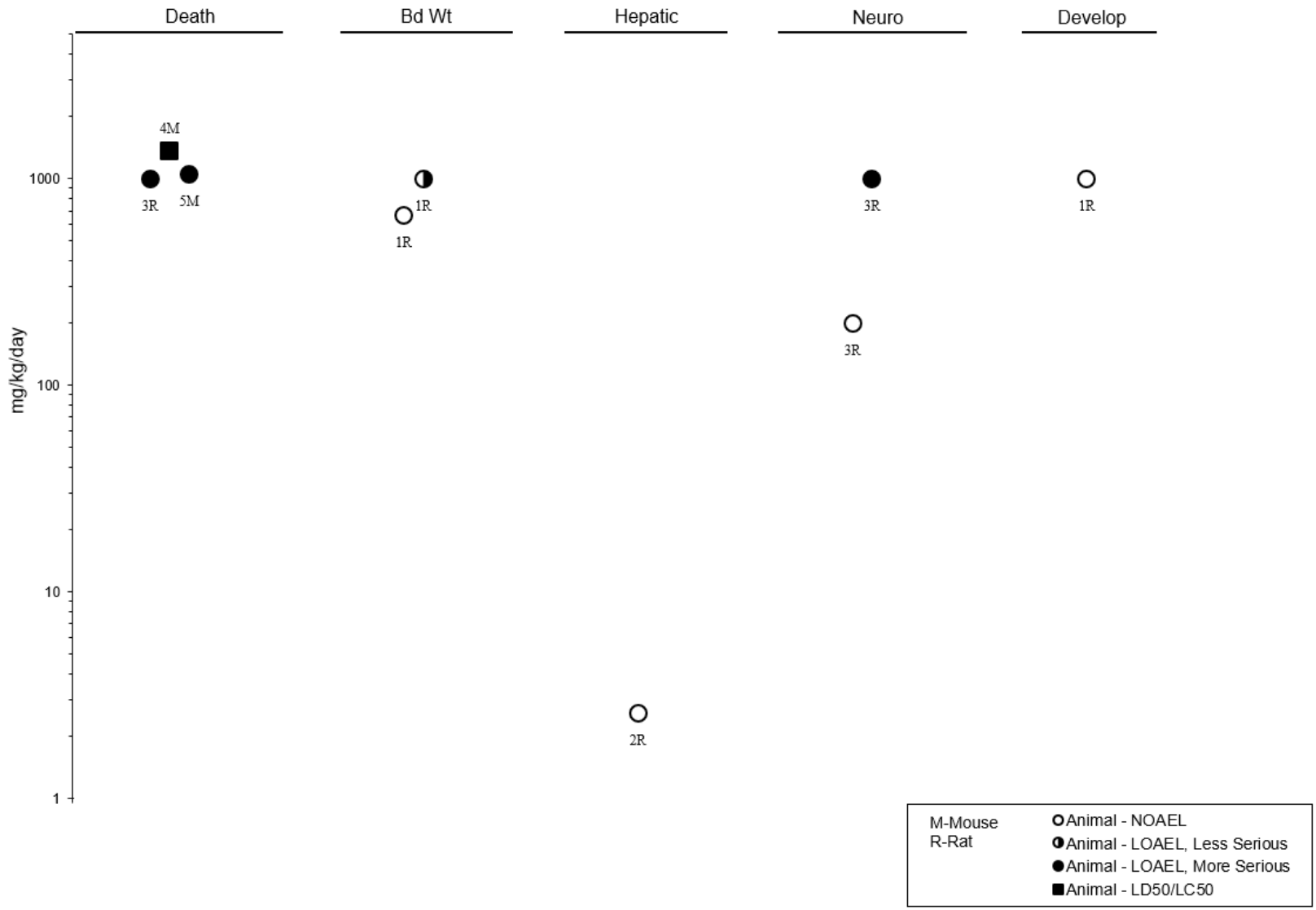


Figure 2-10. Levels of Significant Exposure to 4-Chlorophenol – Oral
Intermediate (15-364 days)

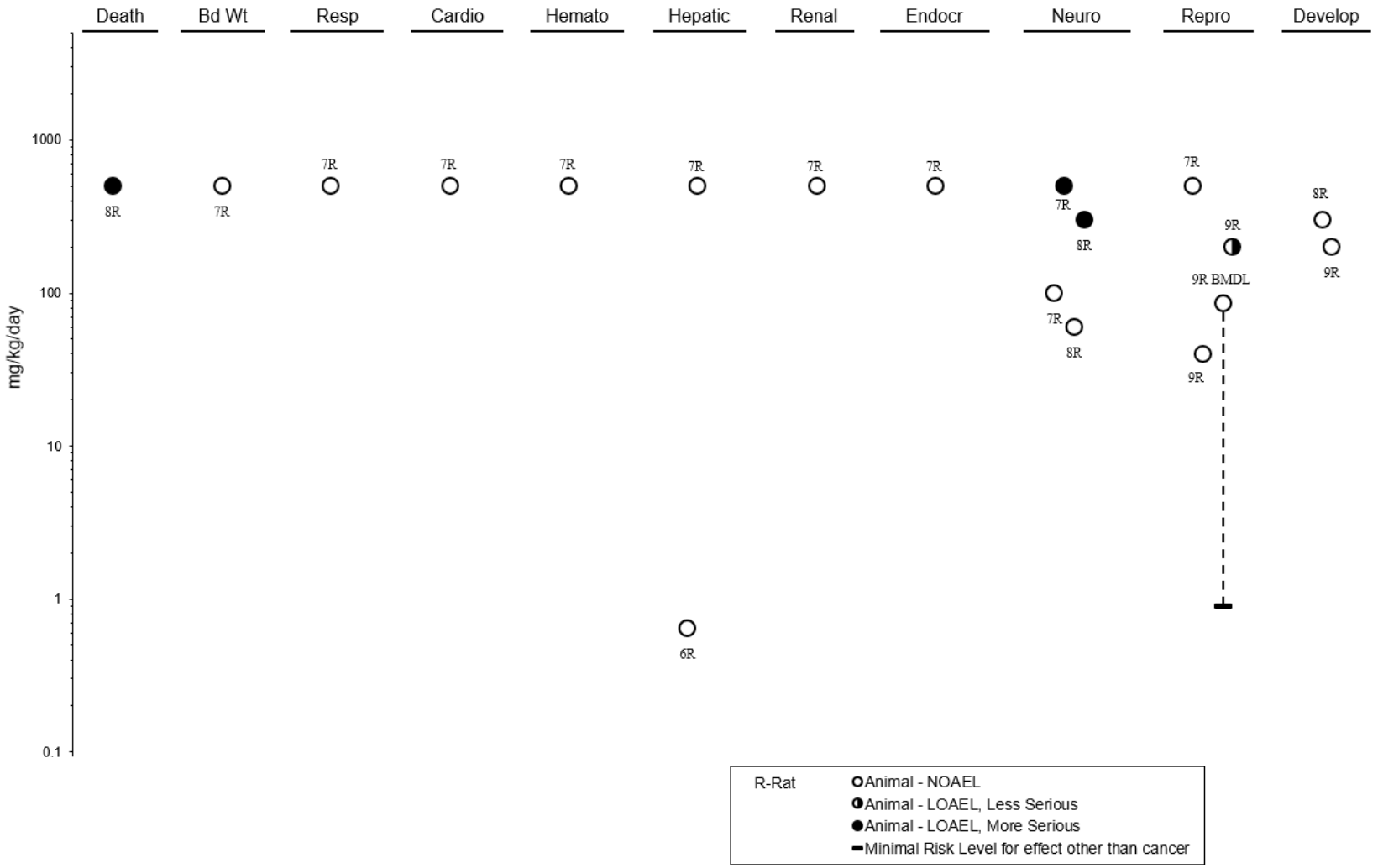


Table 2-4. Levels of Significant Exposure to 2,4-Dichlorophenol – Oral

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)	Effects
ACUTE EXPOSURE									
1	Rat (Fischer 344/N) 5 M, 5 F	14 days (F)	0, 125, 250, 500, 1,000, 2,000	BW, FI, GN, CS, LE	Bd wt	500 M	1,000 M	2,000 M	19% decrease in body weight at 1,000 mg/kg/day; 52% decrease in body weight at 2,000 mg/kg/day
NTP 1989									
2	Rat (Fischer 344) 27–31 F	10 days GDs 6–15 (GO)	0, 200, 375, 750	FX, DX, MX, CS	Death			750 F	4/34 maternal deaths
					Bd wt			375 F	23% decrease in maternal weight gain
					Develop	200	750		Delayed ossification and 3% decrease in fetal body weights
Rodwell et al. 1989									
3	Mouse (CD-1) 8 M, 8 F	Once (GO)	NS	CS	Death			1,276 M	LD_{50}
Borzelleca et al. 1985a, 1985b, 1985c									
4	Mouse (ICR) 6 M, 6 F	2 doses 18 hours apart (GO)	667, 1,000, 1,500, 2,250	LE	Death			1,000	2/12 mice died
Kobayashi et al. 1972									
5	Rat (Sprague-Dawley) 6 M 6 F	2 doses 18 hours apart (GO)	2,000, 2,250, 3,000	LE	Death			1,500	1/12 rats died
Kobayashi et al. 1972									
6	Mouse (B6C3F1) 5 M, 5 F	14 days (F)	0, 325, 650, 1,300, 2,600, 5,200	BW, FI, GN, CS, LE	Death			5,200 M	1/5 deaths
					Bd wt	2,600		5,200 M	25% decreased body weight, reduced food intake
					Neuro	2,600		5,200	Lethargy
NTP 1989									

Table 2-4. Levels of Significant Exposure to 2,4-Dichlorophenol – 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)	Effects
7	Mouse (BALB/c) 6 M/group	14 days (W)	0, 270	BW, OW, BC, HP, OF	Bd wt	270 M			
					Renal	270 M			
					Repro			270 M	Increased necrotic cell counts in seminiferous tubules, >3-fold increase in percent abnormal sperm, and decreased sperm motility
Aydin et al. 2009									
INTERMEDIATE EXPOSURE									
8	Rat (Sprague-Dawley) 10 M, F	Dams: from weaning through mating at PND 90, gestation, and lactation Offspring: from conception through weaning (PND 21) and for additional 15 weeks (W)	0, 0.46, 4.6, 46	BW, DX, RX, OF, OW, HP	Bd wt	46			
					Hemato	46			
					Hepatic	4.6	46		Increased offspring liver weight (19%) at end of exposure
					Immuno	0.46[b]	4.6		Decreased delayed-type hypersensitivity ($BMDL_{1SD}$ = 2.07 mg/kg/day)
					Repro	4.6	46		Decreased litter size
					Develop	46			No effect on birth or weaning weight or survival of offspring to weaning
Exon and Koller 1985; Exon et al. 1984									
9	Rat (Fischer-344/N) 10 M, 10 F	13 weeks (F)	0, 125, 250, 500, 1,000, 2,000	BW, FI, GN, HP, CS, LE	Bd wt	500	1,000 M		20% reduction in body weight
					Resp	2,000			
					Cardio	2,000			
					Gastro	2,000			
					Hemato	250 F		500 F	Bone marrow atrophy in erythroid and myelocytic elements
					Musc/skel	2,000			
					Hepatic	2,000			
					Renal	2,000			
					Dermal	2,000			
					Ocular	2,000			

Table 2-4. Levels of Significant Exposure to 2,4-Dichlorophenol – 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)	Effects
					Endocr	2,000			
					Neuro	1,000	2,000		Hunched posture
NTP 1989									
10	Mouse (CD-1) 20 M, 20 F	90 days (W)	M: 0, 40, 114, 383; F: 0, 50, 143, 491	BW, OW, HP, BC, CS, BI, WI	Bd wt	383			
					Resp	383			
					Hemato	383			
					Hepatic	383			
					Renal	383			
Borzelleca et al. 1985a, 1985c									
11	Mouse (ICR, ddN) 10 M	6 months (F)	0, 45, 100, 230	BW, FI, HP, CS, BC, OW	Bd wt	230 M			
					Cardio	230 M			
					Hemato	230 M			
					Hepatic	100 M	230 M		Hepatocyte swelling
					Renal	230 M			
Kobayashi et al. 1972									
12	Mouse (B6C3F1) 10 M, 10 F	3 weeks (F)	0, 325, 650, 1,300, 2,600, 5,200	CS, LE	Death			5,200	20/20 died within 3 weeks
NTP 1989									
13	Mouse (B6C3F1) 10 M, 10 F	13 weeks (F)	0, 325, 650, 1,300, 2,600, 5,200	BW, FI, GN, HP, CS	Bd wt	1,300	2,600		10–15% reduction in body weight
					Resp	2,600			
					Cardio	2,600			
					Gastro	2,600			
					Hemato	2,600			
					Musc/skel	2,600			
					Hepatic		325 M	2,600 M	Minimal hepatocellular necrosis in 4/10 at 325 mg/kg/day; hepatocellular necrosis in 10/10 at 2,000 mg/kg/day
					Renal	2,600			

Table 2-4. Levels of Significant Exposure to 2,4-Dichlorophenol – 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)	Effects
					Dermal	2,600			
					Ocular	2,600			
					Endocr	2,600			
					Neuro	2,600			
NTP 1989									
14	Mouse (CD-1) 4 M	90 days (W)	0, 50, 150, 500	OF	Repro	500 M			No adverse effect on sperm motility or acrosome integrity, or ovum penetration
Seyler et al. 1984									
15	Rat (Wistar) 24/sex	28 weeks (3 generations: 10 weeks premating through gestation and lactation until weaning of 3rd generation) (F)	M: 0, 33.4, 134, 543; F: 0, 49.1, 194, 768	CS, FI, BW, RX, DX, GN, OW, BC, HP	Bd wt	134	543 M		Decreased body weights in females in P generation (6%), and males and females (8 and 13%, respectively) of the F1 generation
					Repro	768			
					Develop	194	768 F		In offspring generations (F1, F2), increased uterine weight (42%) and increased height of epithelial cells of the uterus, as well as, increased uterine growth; 12% reduction in the time to vaginal opening; reduced percentage of pups with eye opening on day 14
Aoyama et al. 2005									
CHRONIC EXPOSURE									
16	Rat (Sprague-Dawley) 10 M, F	From conception through weaning (PND 21) and for additional 10–15 weeks (W)	0, 0.44, 4.4, 44	HE, HP	Hemato	37			
Exon and Koller 1985; Exon et al. 1984									
17					Bd wt	120 F	250 F		6–12% reduced body weight

Table 2-4. Levels of Significant Exposure to 2,4-Dichlorophenol – 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)	Effects
	Rat (Fischer 344) 50 F, 50 M	103 weeks (F)	F: 0, 120, 250; M: 0, 210, 440	BW, FI, GN, HP, CS, LE	Resp			210 M	Nasal lesions; multifocal degeneration of respiratory epithelium
					Cardio	440 M			
					Gastro	440 M			
					Hemato	440 M			
					Musc/skel	440 M			
					Hepatic	440 M			
					Renal	440 M			
					Dermal	440 M			
					Ocular	440 M			
					Endocr	440 M			
					Immuno	440 M			
					Neuro	440 M			
					Repro	250 F			
						440 M			
NTP 1989									
18	Mouse (B6C3F1) 50 F, 50 M	103 weeks (F)	F: 0, 430, 820; M: 0, 800, 1,300	BW, FI, GN, HP, CS	Bd wt	430 F	820 F		Maximum 19% decrease in body weight relative to controls
					Resp	1,300 M			
					Cardio	1,300 M			
					Gastro	1,300 M			
					Hemato	1,300 M			
					Musc/skel	1,300 M			
					Hepatic	1,300 M			
					Renal	1,300 M			
					Dermal	1,300 M			
					Ocular	1,300 M			
					Endocr	1,300 M			
					Immuno	1,300 M			

Table 2-4. Levels of Significant Exposure to 2,4-Dichlorophenol – 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)	Effects
					Neuro	1,300 M			
					Repro	820 F			
						1,300 M			
NTP 1989									

[a]The number corresponds to entries in Figure 2-11; differences in levels of health effects and cancer effects between male and females are not indicated in the figure. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an intermediate-duration oral MRL of 0.02 mg/kg/day using BMD analysis. The $BMDL_{1SD}$ of 2.07 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability). See Appendix A for details.

BC = blood chemistry; Bd wt or BW = body weight; BI = biochemical changes; BMD = benchmark dose; BMDL = benchmark dose, lower confidence limit; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental effects; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; FX = fetal toxicity; (GO) = gavage in oil; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; Hemato = hematological; HP = histopathology; Immuno = immunological; LD_{50} = dose producing 50% death; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; Musc/skel = muscular/skeletal; MX = maternal toxicity; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function; OW = organ weight; PND = postnatal day; Repro = reproductive; Resp = respiratory; RX = reproductive effects; SD = standard deviation; (W) = water; WI = water intake

Figure 2-11. Levels of Significant Exposure to 2,4-Dichlorophenol – Oral
Acute (≤14 days)

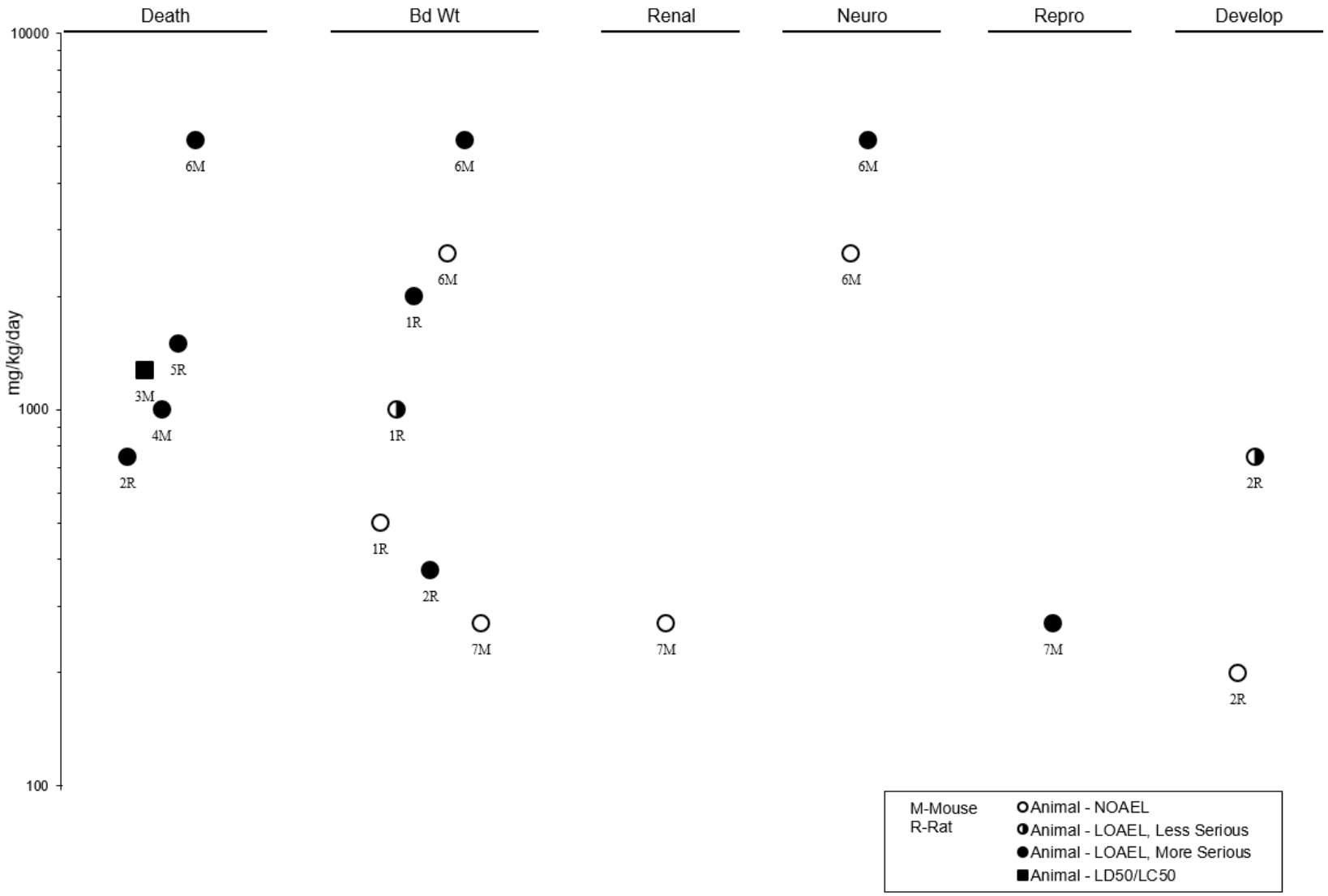


Figure 2-11. Levels of Significant Exposure to 2,4-Dichlorophenol – Oral
Intermediate (15-364 days)

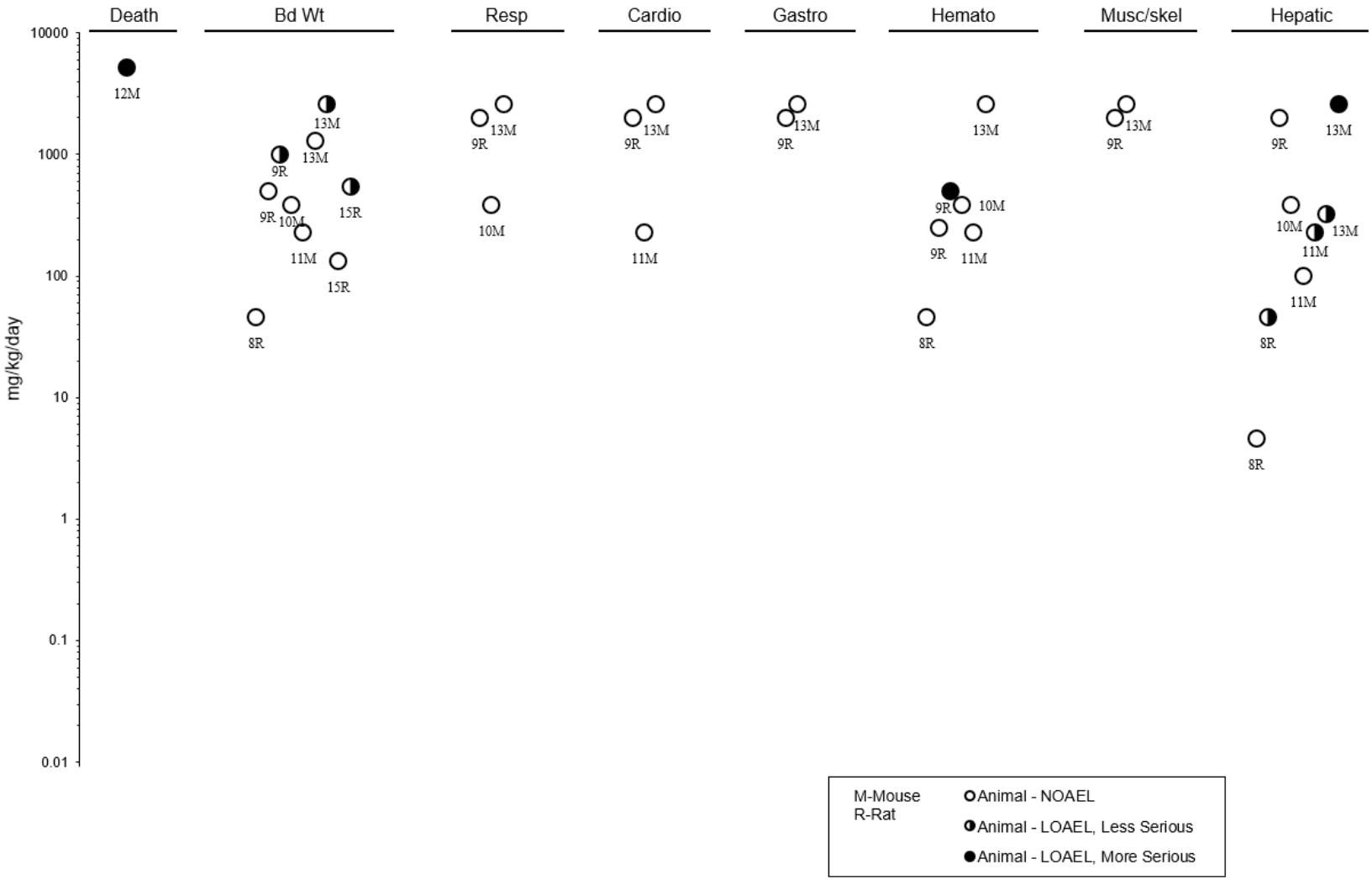


Figure 2-11. Levels of Significant Exposure to 2,4-Dichlorophenol – Oral
Intermediate (15-364 days)

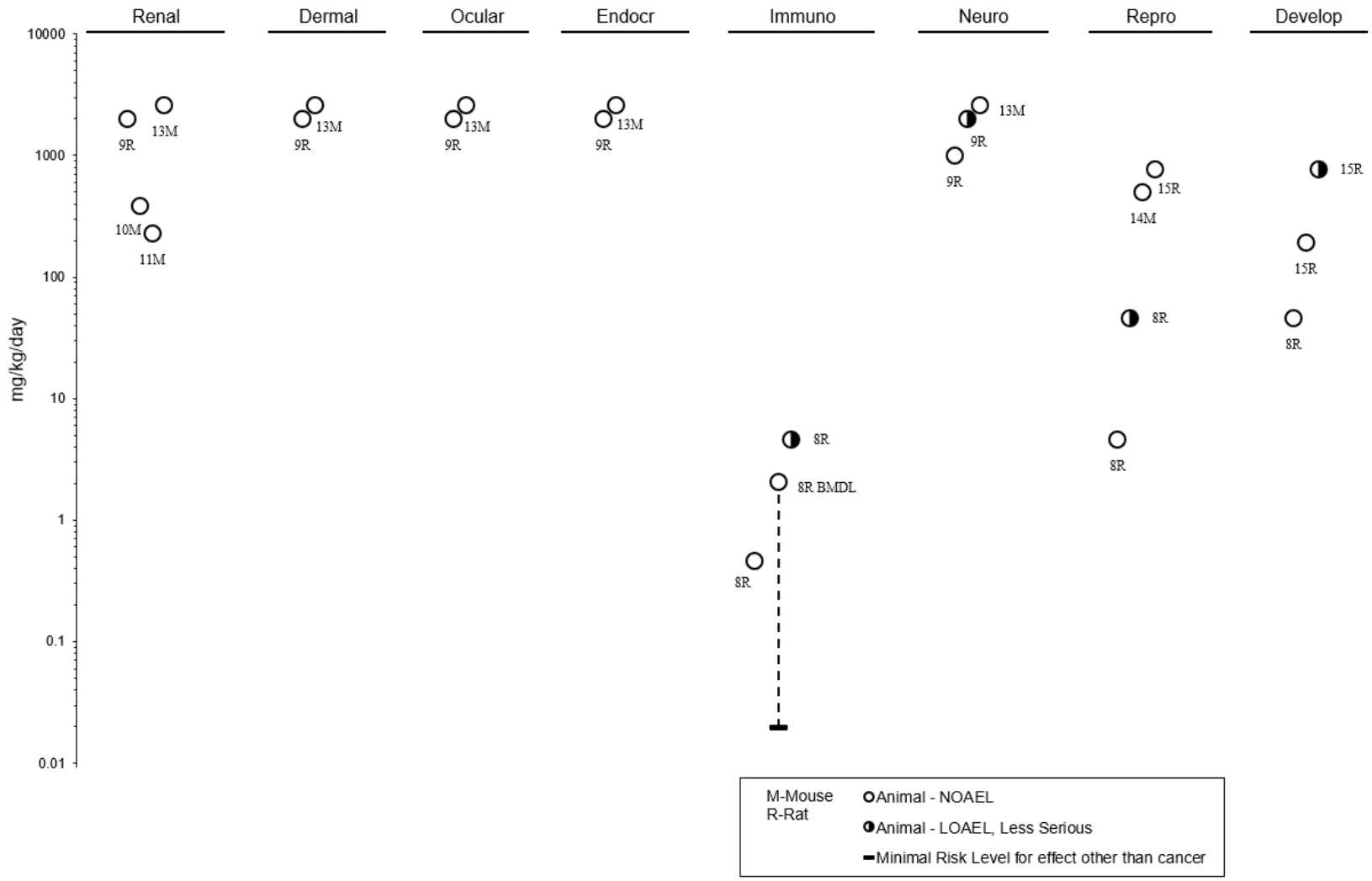


Figure 2-11. Levels of Significant Exposure to 2,4-Dichlorophenol – Oral

Chronic (≥365 days)

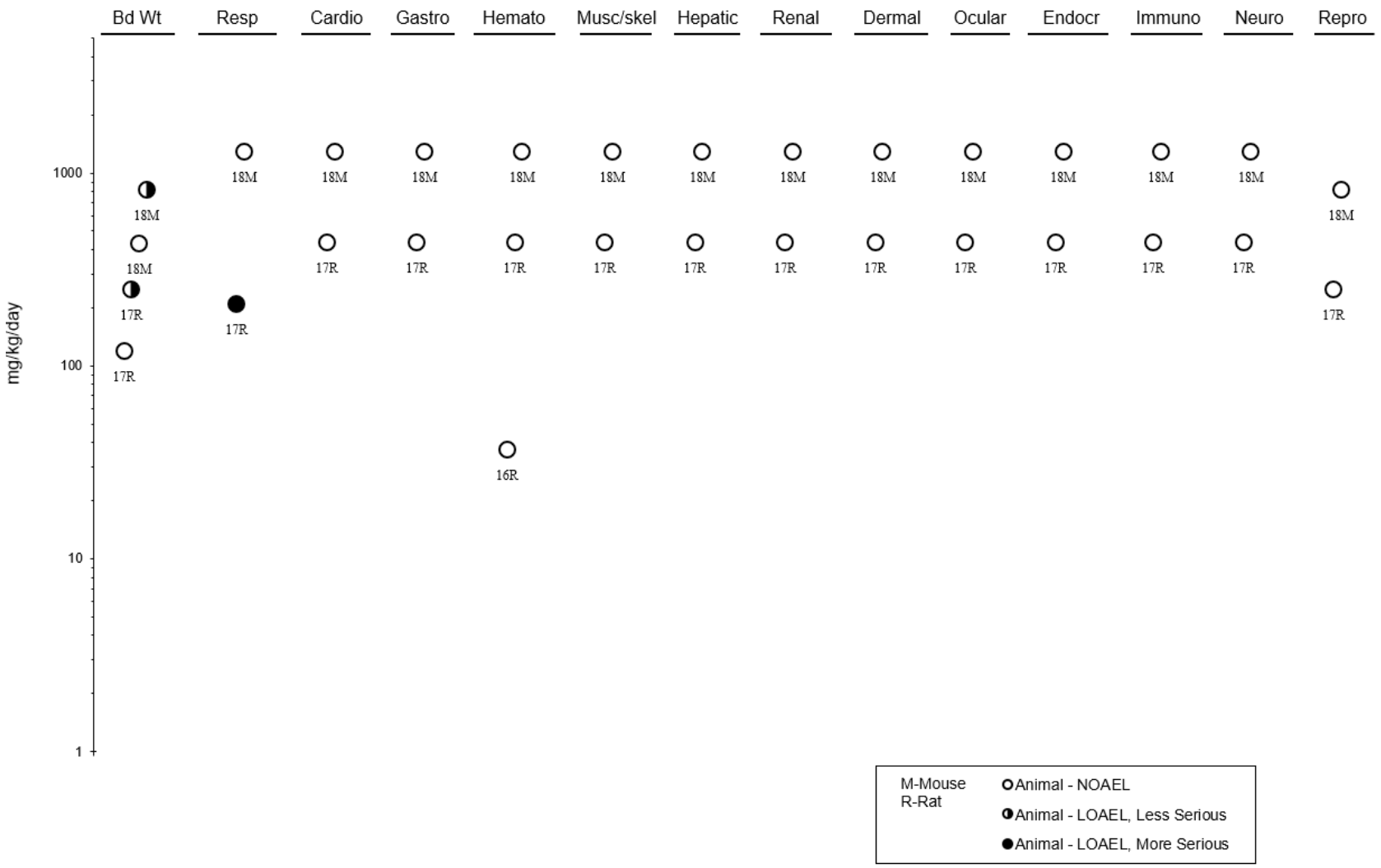


Table 2-5. Levels of Significant Exposure to 2,4,5-Trichlorophenol – Oral

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)	Effects
ACUTE EXPOSURE									
1	Rat (NS) 5 M	Once (GO)	1,000, 1,260, 1,580, 2,000, 2,520, 3,160, 3,980	LE	Death			2,960 M	LD_{50}
McCollister et al. 1961									
2	Rat (Sprague-Dawley) 6 M	14 days (GO)	0, 25, 100, 400	EA	Hepatic	400			No effect on hepatic enzyme levels
Carlson 1978									
INTERMEDIATE EXPOSURE									
3	Rat (Wistar) 10 M, 10 F	98 days (F)	0, 10, 30, 100, 300, 1,000	CS, BW, FI, HE, BC, OW, GN, HP	Bd wt	300 F		1,000 F	10% decrease in terminal body weight
					Resp	1,000			
					Cardio	1,000			
					Hemato	1,000			
					Hepatic	100[b]	300		Mild centrilobular degeneration

Table 2-5. Levels of Significant Exposure to 2,4,5-Trichlorophenol – 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)	Effects
					Renal	100[b]	300		Slight degenerative changes in convoluted tubule epithelium
					Repro	1,000			No effect on testes weight or histology
					Endocr	1,000			
McCollister et al. 1961									

[a]The number corresponds to entries in Figure 2-12; differences in levels of health effects and cancer effects between male and females are not indicated in the figure. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an intermediate-duration oral MRL of 1.0 mg/kg/day. The NOAEL of 100 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability). See Appendix A for details.

Bd wt or BW = body weight; BC = biochemistry; Cardio = cardiovascular; CS = clinical signs; Endocr = endocrine; EA = enzyme activity; (F) = feed; F = female(s); FI = food intake; FX = fetal toxicity; (GO) = gavage in oil; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; LD_{50} = dose producing 50% lethality; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; NOAEL = no-observed-adverse-effect level; NS = not specified; OW = organ weight; Repro = reproductive; Resp = respiratory

Figure 2-12. Levels of Significant Exposure to 2,4,5-Trichlorophenol – Oral

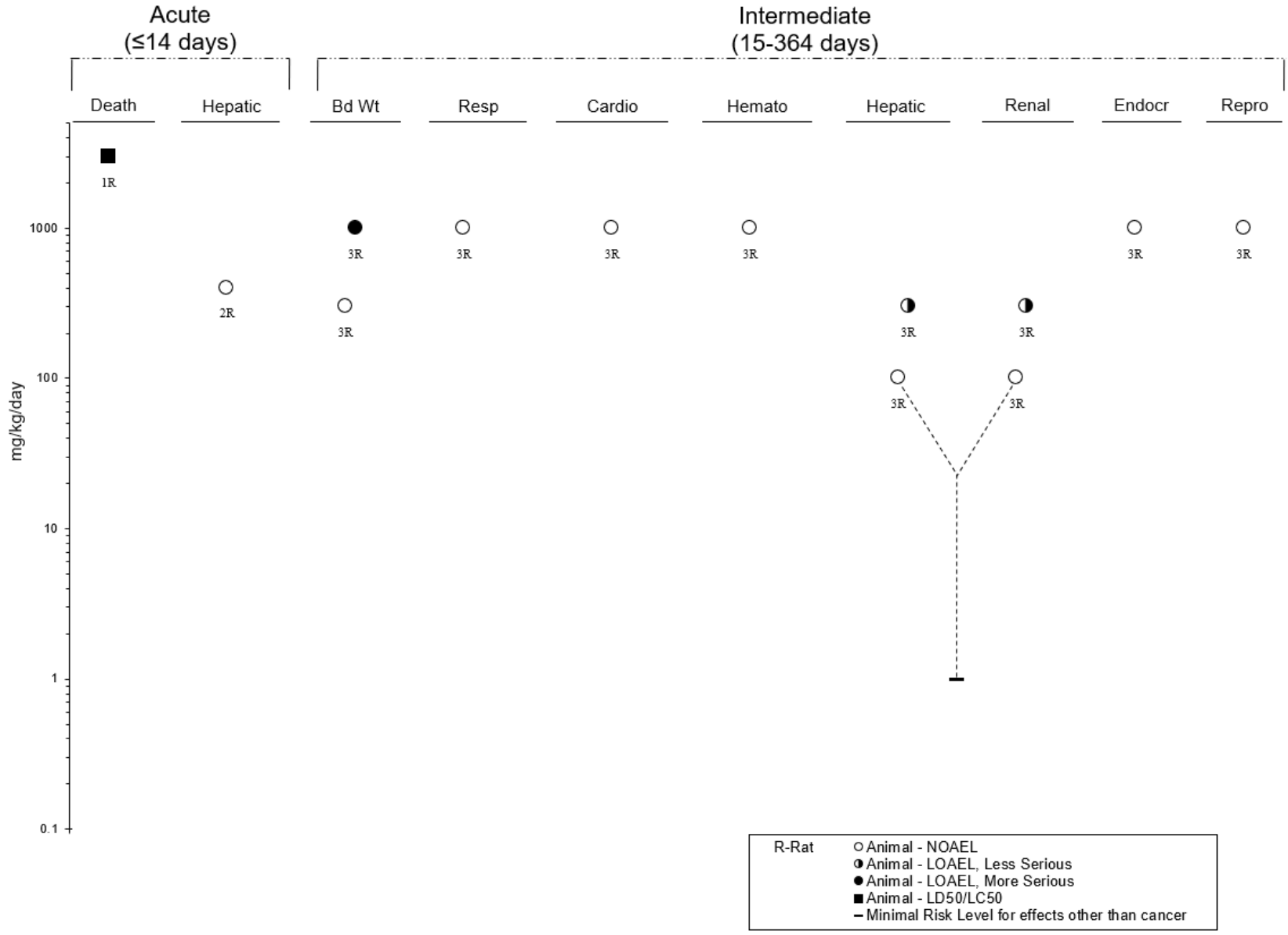


Table 2-6. Levels of Significant Exposure to 2,4,6-Trichlorophenol – Oral

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)	Effects
ACUTE EXPOSURE									
1	Rat (Sprague-Dawley) 6 M	14 days (GO)	0, 25, 100, 400	BI, OF	Hepatic	400			No effect on hepatic enzyme activities
Carlson 1978									
INTERMEDIATE EXPOSURE									
2	Rat (Long-Evans hooded) 30 or 40 F	2 weeks 5 days/week; and GDs 1–21, 7 days/week (GO)	0, 100, 500, 1,000	BW, LE, CS	Bd wt	500	1,000		Reduced mean maternal body weight
					Develop	100	500		10–11% reduction in litter weight
Blackburn et al. 1986									
3	Rat (Sprague-Dawley) 10 M, 10 F	90 days (GO)	0, 80, 240, 720	CS, BW, FI, BC, OP, HE, UR, OW, GN, HP	Bd wt	720			
					Resp	720			
					Cardio	720			
					Gastro	720			
					Hemato	720			
					Hepatic	80	240 M		14% increased relative liver weight
					Renal	240	720 M		Increased kidney weight, decreased urinary pH
					Ocular	720			
					Endocr	720			
Bercz et al. 1990									

Table 2-6. Levels of Significant Exposure to 2,4,6-Trichlorophenol – 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)	Effects
4	Rat (Long-Evans hooded) 15–25 M	11 weeks 5 days/week (GO)	0, 100, 500, 1,000	CS, LE, RX, OW, BW, GN	Death			1,000 M	8/25 died
					Bd wt	1,000 M			
					Resp	1,000 M			
					Cardio	1,000 M			
					Hepatic	1,000 M			
					Renal	1,000 M			
					Endocr	1,000 M			
					Repro	1,000 M			
Blackburn et al. 1986									
5	Rat (Fischer 344) 5 M, 5 F	7 weeks, 7 days/week (F)	0, 500, 735, 1,075, 1,575, 2,300	BW, LE, HP	Bd wt	500	735	1,075	11–16% decrease in body weight at 735 mg/kg/day; 27% decrease in body weight at 1,075 mg/kg/day
					Hemato	1,575	2,300		Increased splenic hematopoiesis
					Hepatic	1,575	2,300 M		Midzonal vacuolation of hepatocytes
NCI 1979									
6	Rat (Sprague-Dawley) 10–14 NS	Dams: from weaning through mating at PND 90, gestation, and lactation Offspring: from conception through weaning (PND 21) and for additional 15 weeks (W)	0, 0.46, 4.6, 46	BW, OW	Bd wt	46			
					Hemato	46			
					Hepatic	0.46[b]	4.6		Increased liver weight (15%)
					Immuno	4.6	46		Increased spleen weight
					Repro	4.6	46		Decreased mean litter size
					Develop	46			No effect on birth or weaning weight or survival to weaning
Exon and Koller 1985									

Table 2-6. Levels of Significant Exposure to 2,4,6-Trichlorophenol – 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)	Effects
7	Mouse (B6C3F1) 5 M, 5 F	7 weeks 7 days/week (F)	0, 884, 1,300, 1,911, 2,795, 4,095	HP, BW, GN, CS, LE	Death			4,095	4/10 died
NCI 1979									
CHRONIC EXPOSURE									
8	Rat (Fischer 344) 50 M, 50 F	107 weeks 7 days/weeks (F)	0, 250, 500	BW, GN, HP, CS, LE	Bd wt		250 F	500 F	Approximate 10% decrease in body weight at 250 mg/kg/day; approximate 29% decrease in body weight at 500 mg/kg/day
					Resp	500			
					Cardio	500			
					Gastro	500			
					Hemato			250 M	Bone marrow hyperplasia
					Hepatic	500			
					Renal	500			
					Dermal	500			
					Endocr	500			
					Immuno	500			
					Neuro	500			
					Repro	500			
					Cancer			250 M	CEL: monocytic leukemia 23/50
NCI 1979									
9	Mouse (B6C3F1) 50 M, 50 F	105 weeks 7 days/week (F)	M: 0, 650, 1,300; F: 0, 678, 1,356 (TWA)	BW, GN, HP, CS	Bd wt			658 F	Approximately 24% decrease in body weight
					Resp	1,300 M			
					Cardio	1,300 M			
					Gastro	1,300 M			
					Hemato	1,300 M			
					Hepatic			650 M	Hepatic hyperplasia

Table 2-6. Levels of Significant Exposure to 2,4,6-Trichlorophenol – 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)	Effects
					Renal	1,356			
					Dermal	1,356			
					Endocr	1,356			
					Neuro	1,356 F			
					Repro	1,356 F			
						1,300 M			
					Cancer			650 M	CEL: 7/47 hepatocellular carcinomas or adenomas
NCI 1979									

[a]The number corresponds to entries in Figure 2-13; differences in levels of health effects and cancer effects between male and females are not indicated in the figure. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an intermediate-duration oral MRL of 0.005 mg/kg/day (5 µg/kg/day). The NOAEL of 0.46 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). See Appendix A for details.

BC = blood chemistry; Bd wt or BW = body weight; BI = biochemical changes; BMD = benchmark dose; BMDL = benchmark dose, lower confidence limit; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental effects; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; (GO) = gavage in oil; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; 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; NS = not specified; OF = organ function; OP = ophthalmology; OW = organ weight; PND = postnatal day; Repro = reproductive; Resp = respiratory; RX = reproductive effects; SD= standard deviation; TWA = time-weighted average; UR = urinalysis; (W) = water

Figure 2-13. Levels of Significant Exposure to 2,4,6-Trichlorophenol – Oral

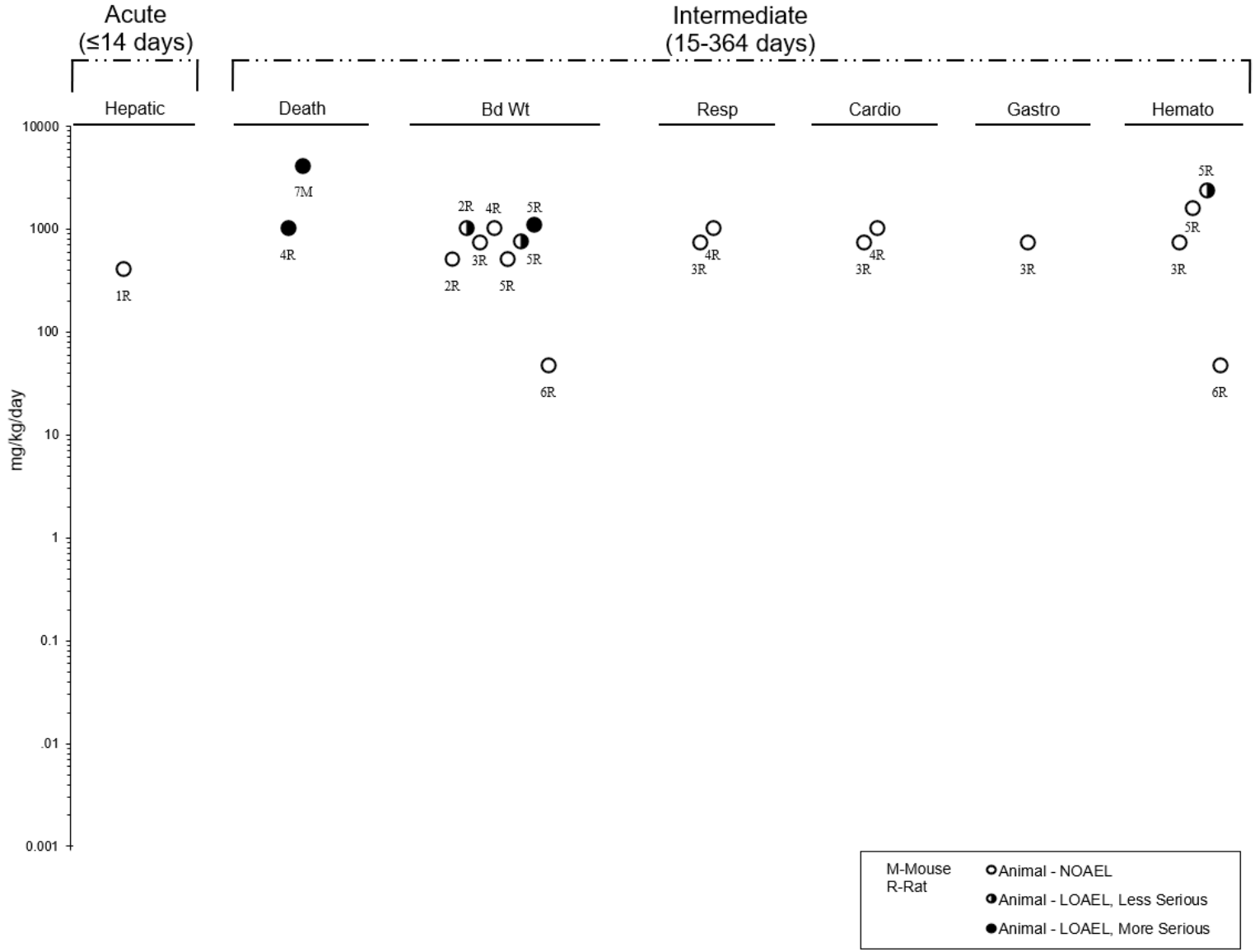


Figure 2-13. Levels of Significant Exposure to 2,4,6-Trichlorophenol – Oral
Intermediate (15-364 days)

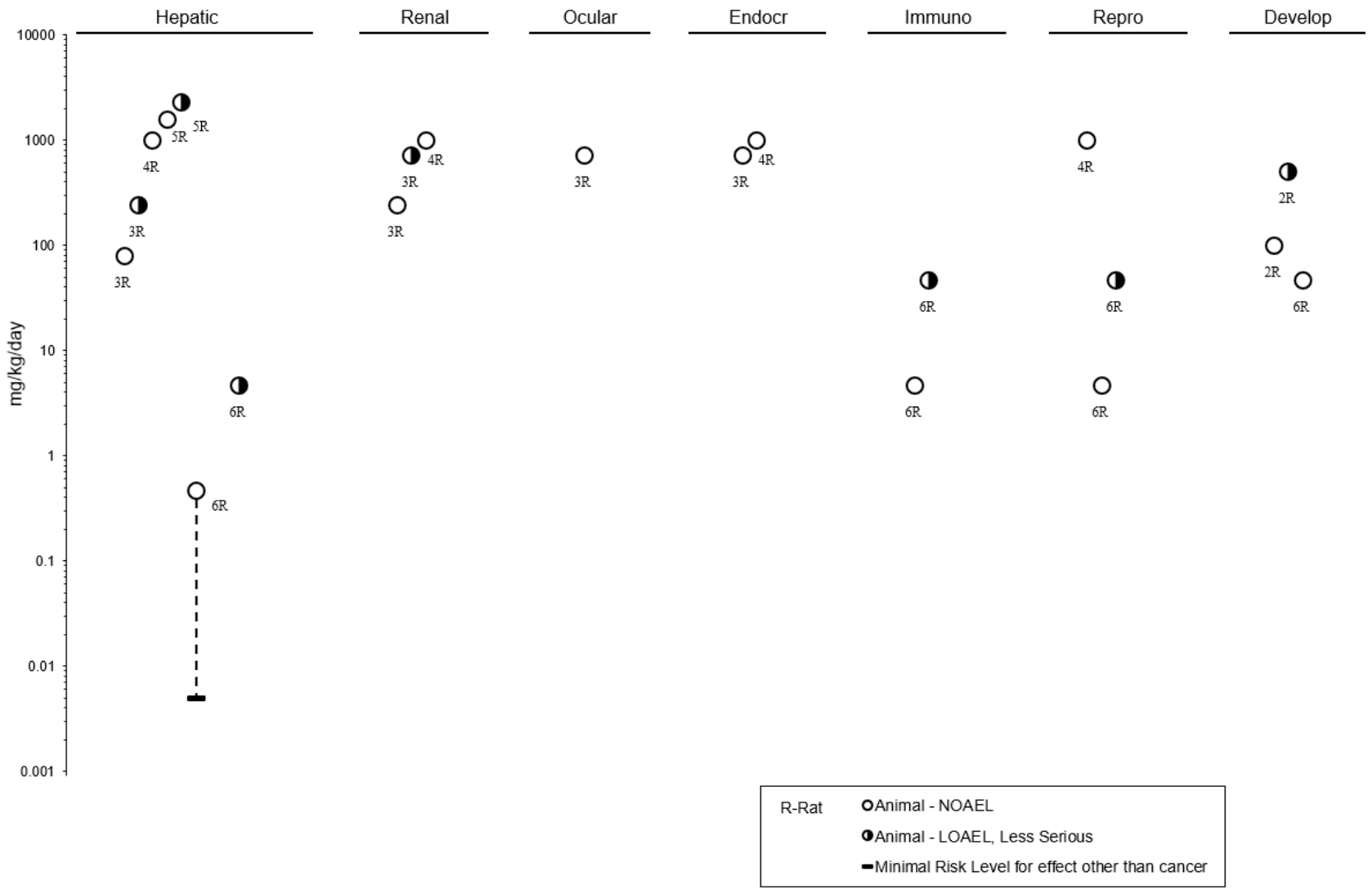


Figure 2-13. Levels of Significant Exposure to 2,4,6-Trichlorophenol – Oral
Chronic (≥365 days)

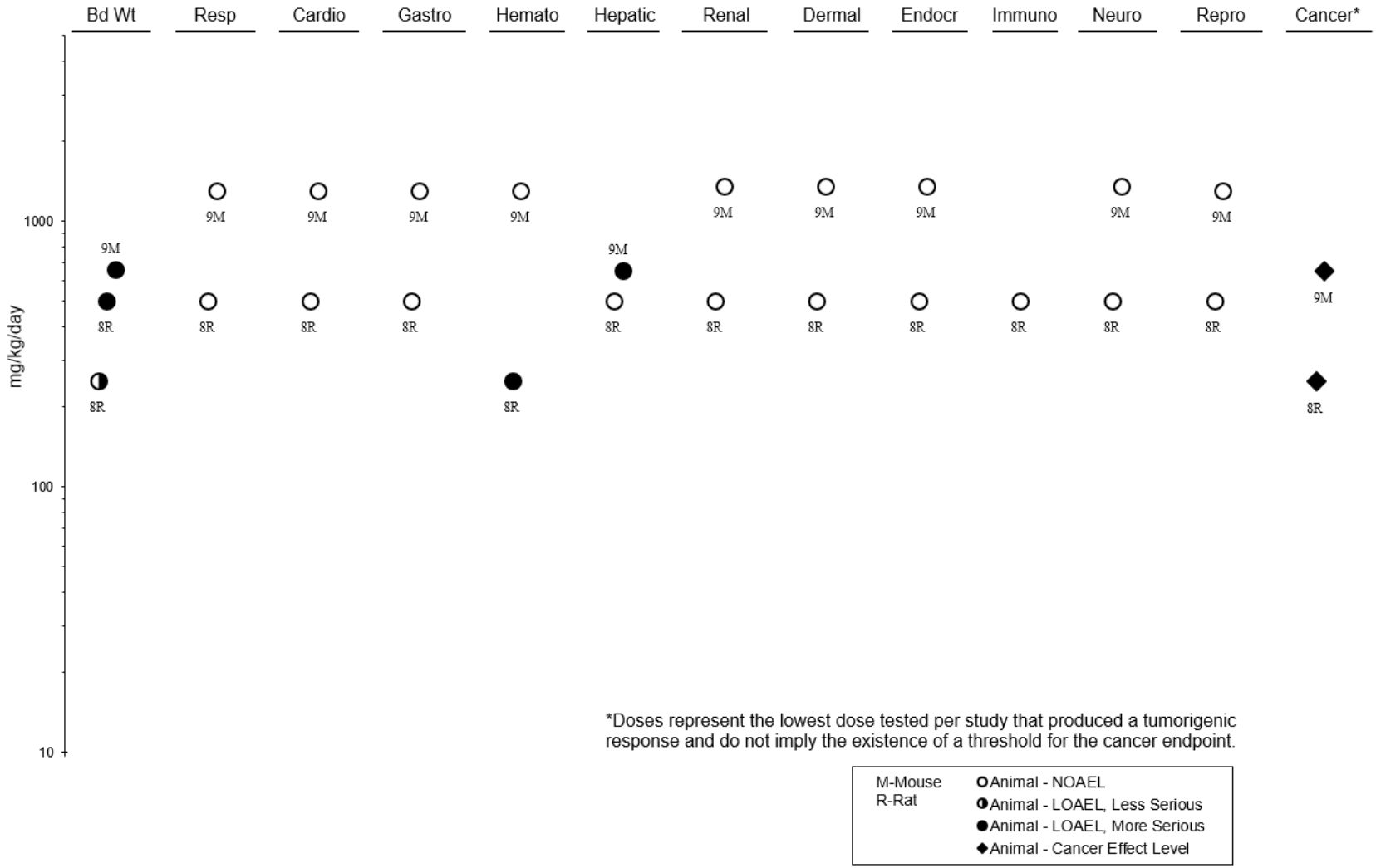


Table 2-7. Levels of Significant Exposure to 2,3,4,6-Tetrachlorophenol – Oral

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)	Effects
ACUTE EXPOSURE									
1	Gerbil (NS) NS F	Once (G)	NS	LE	Death			698 F	LD_{50}
Ahlborg and Larsson 1978									
2	Rat (Wistar) 10 NS	Once (GO)	0, 300, 360, 410, 432, 518, 632	HP	Gastro	410	432	632	Mild necrosis at 432 mg/kg/day; mucosal hyperemia of stomach, severe necrosis of intestine at 632 mg/kg/day
					Musc/skel	632			
					Renal	632			
Hattula et al. 1981									
3	Rat (CD) 18–22 F	GDs 6–15 (GO)	0, 25, 100, 200	BW, FX, DX, MX, FI, GN	Bd Wt	25 F	100 F	200 F	Decrease in corrected maternal body weight gain: 13% at 100 mg/kg/day; 26% at 200 mg/kg/day
					Develop	200			
EPA 1987a, 1987b									
4	Rat (Sprague-Dawley) 10 M	5 days (GO)	0, 10, 25, 50, 100, 200	CS, BW, BC, OW, HP	Bd wt	200 M			
					Hepatic	100 M	200 M		23 and 26% increases in absolute and relative liver weights; increased incidence of centrilobular hypertrophy and low incidence of necrosis
Dodd et al. 2012									
5	Rat (Sprague-Dawley) 10 M	2 weeks, 7 days/week (GO)	0, 10, 25, 50, 100, 200	CS, BW, BC, OW, HP	Bd wt	200 M			
					Hepatic	10[b] M	25 M		15 and 14% increases in absolute and relative liver weights; low incidence of vacuolation ($BMDL_{1SD}$ = 8.45 mg/kg/day)
Dodd et al. 2012									
6	Mouse (C57 black) 4 M, 4 F	Once (G)	NS	LE	Death			131 F	LD_{50}
Ahlborg and Larsson 1978									

Table 2-7. Levels of Significant Exposure to 2,3,4,6-Tetrachlorophenol – 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)	Effects
INTERMEDIATE EXPOSURE									
7	Rat (Wistar) NS	55 days, 7 days/week (GO)	0, 10, 50, 100	HP, BW, FI, HE	Bd wt	100			
					Gastro	50	100		Focal necrosis of small intestine
					Musc/skel	100			
					Hepatic	10		50	Necrosis, thrombosed veins
Hattula et al. 1981									
8	Rat (Sprague-Dawley) 245 M (10/group)	13 weeks, 7 days/week (GO)	0, 10, 25, 50, 100, 200	CS, BW, BC, OW, GN, HP	Bd wt	50 M	100 M		12% decrease in body weight
					Hepatic		10[c] M		27 and 18% increases in absolute and relative liver weights; increased incidences and/or severity of centrilobular vacuolation and hypertrophy ($BMDL_{10}$ = 1.02 mg/kg/day)
Dodd et al. 2012									
9	Rat (Sprague-Dawley) 10 M	4 weeks, 7 days/week (GO)	0, 10, 25, 50, 100, 200	CS, BW, BC, OW, HP	Bd wt	200 M			
					Hepatic	10 M	25 M		Increased incidences of hepatic centrilobular vacuolation and hypertrophy
Dodd et al. 2012									
10	Rat (Sprague-Dawley) 30 M, 30 F	90 days, 7 days/week (GO)	0, 25, 100, 200	LE, HP, CS, BW, FI, OW, GN, BI, HE	Bd wt	100	200 M		Body weight gain decreased by 11%
					Resp	200			
					Cardio	200			
					Gastro	200			
					Hemato	200			
					Musc/skel	200			
					Hepatic	25	100		Increased liver weights and centrilobular hypertrophy
					Renal	25	100		Increased kidney weights
					Ocular	200			

Table 2-7. Levels of Significant Exposure to 2,3,4,6-Tetrachlorophenol – 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)	Effects
					Endocr	200			
					Immuno	200			
					Neuro	200			
					Repro	200			

EPA 1986 (formerly cited as American Biogenics)

[a]The number corresponds to entries in Figure 2-14; differences in levels of health effects and cancer effects between male and females are not indicated in the figure. 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 of 0.08 mg/kg/day using BMD analysis. The $BMDL_{1SD}$ of 8.45 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). See Appendix A for details.
[c]Used to derive an intermediate-duration oral MRL of 0.01 mg/kg/day using BMD analysis. The $BMDL_{10}$ of 1.02 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). See Appendix A for details.

BC = blood chemistry; Bd wt or BW = body weight; BI = biochemical changes; BMD = benchmark dose; BMDL = benchmark dose, lower confidence limit; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental effects; Endocr = endocrine; F = female(s); FI = food intake; FX = fetal toxicity; (G) = gavage; (GO) = gavage in oil; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; LD_{50} = dose producing 50% death; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = muscular/skeletal; MX = maternal toxicity; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OW = organ weight; Repro = reproductive; Resp = respiratory; SD = standard deviation

Figure 2-14. Levels of Significant Exposure to 2,3,4,6-Tetrachlorophenol – Oral
Acute (≤14 days)

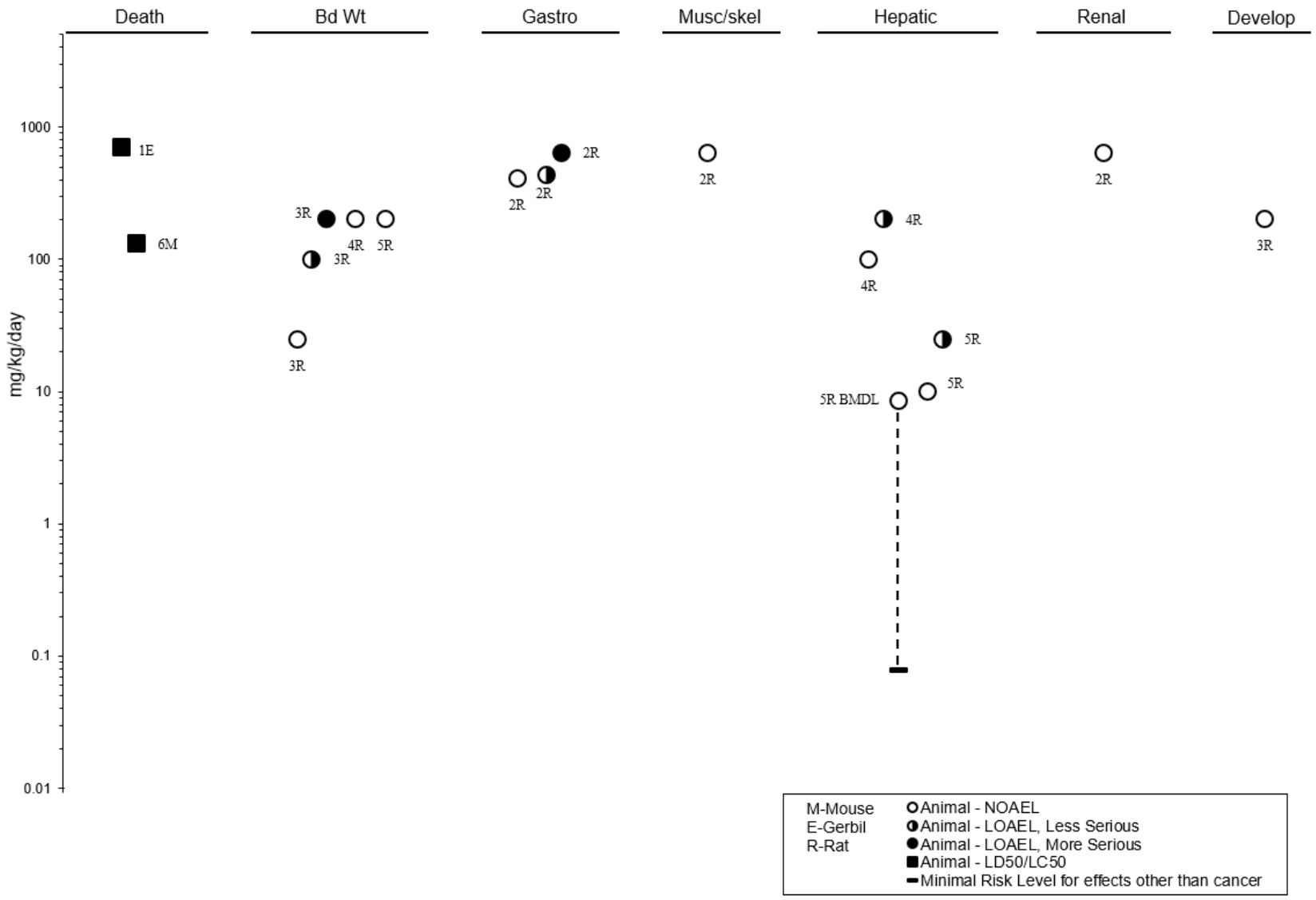


Figure 2-14. Levels of Significant Exposure to 2,3,4,6-Tetrachlorophenol – Oral
Intermediate (15-364 days)

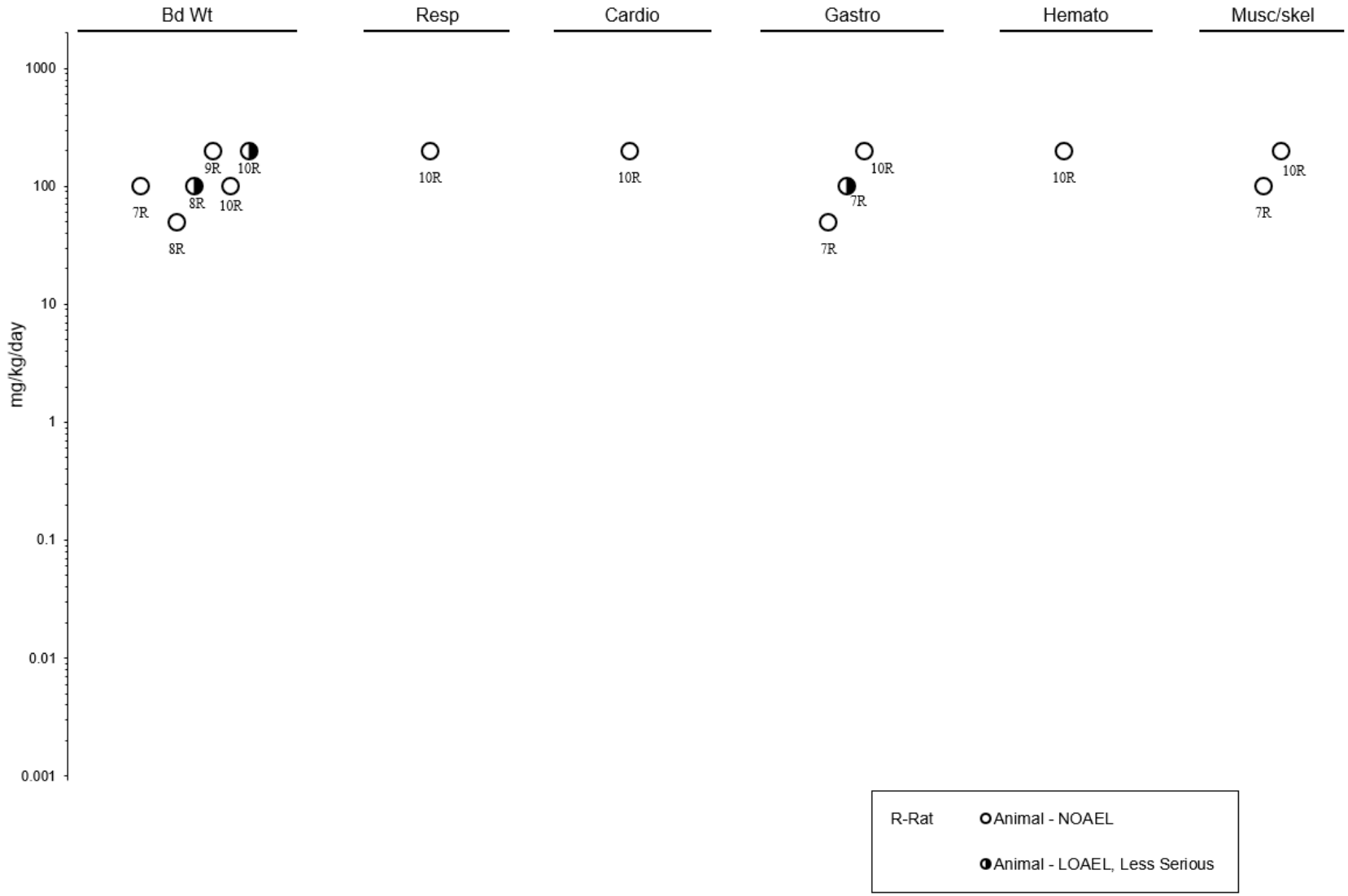


Figure 2-14. Levels of Significant Exposure to 2,3,4,6-Tetrachlorophenol – Oral
Intermediate (15-364 days)

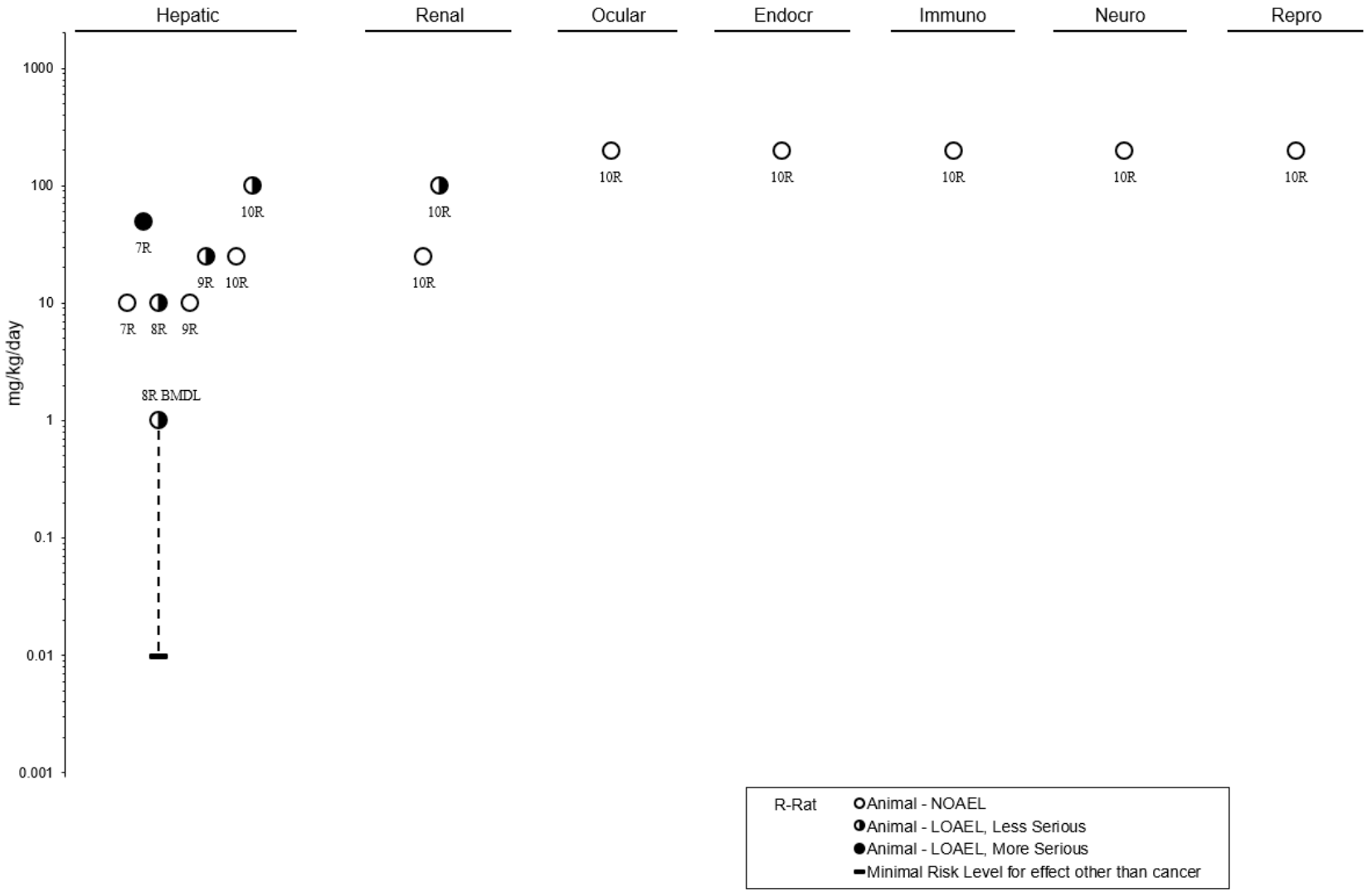


Table 2-8. Levels of Significant Exposure to Other Chlorophenols – Oral

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)	Effects
ACUTE EXPOSURE									
1	Mouse (CD-1) 8 M, 8 F	Once (GO)	NS	LE	Death			2,376 F	LD_{50}
2,3-Dichlorophenol Borzelleca et al. 1985b									
2	Mouse (CD-1) 8 M, 8 F	Once (GO)	NS	LE	Death			946 F	LD_{50}
2,5-Dichlorophenol Borzelleca et al. 1985b									
3	Mouse (CD-1) 8 M, 8 F	Once (GO)	NS	LE	Death			1,685 M	LD_{50}
3,4-Dichlorophenol Borzelleca et al. 1985b									
4	Mouse (CD-1) 8 M, 8 F	Once (GO)	NS	CS	Death			2,389 F	LD_{50}
3,5-Dichlorophenol Borzelleca et al. 1985b									
5	Gerbil (NS) NS F	Once (G)	NS	LE	Death			979 F	LD_{50}
2,3,5,6-Tetrachlorophenol Ahlborg and Larsson 1978									
6	Gerbil (NS) NS F	Once (G)	NS	LE	Death			533 F	LD_{50}
2,3,4,5-Tetrachlorophenol Ahlborg and Larsson 1978									

Table 2-8. Levels of Significant Exposure to Other Chlorophenols – 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)	Effects
7	Mouse (C57 black) 4 F, 4 M	Once (G)	NS	LE	Death			89 M	LD_{50}
2,3,5,6-Tetrachlorophenol Ahlborg and Larsson 1978									
8	Mouse (C57 black) 4 M, 4 F	Once (G)	NS	LE	Death			400 F	LD_{50}
2,3,4,5-Tetrachlorophenol Ahlborg and Larsson 1978									

[a]The number corresponds to entries in Figure 2-15; differences in levels of health effects and cancer effects between male and females are not indicated in the figure. Where such differences exist, only the levels of effect for the most sensitive sex are presented.

Bd wt or BW = body weight; CS = clinical signs; Develop = developmental; DX = developmental effects; (F) = feed; F = female(s); FX = fetal toxicity; (G) = gavage; (GO) = gavage in oil; GD = gestation day; LD_{50} = dose producing 50% lethality; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; NS = not specified

Figure 2-15. Levels of Significant Exposure to Other Chlorophenols – Oral
Acute (≤14 days)

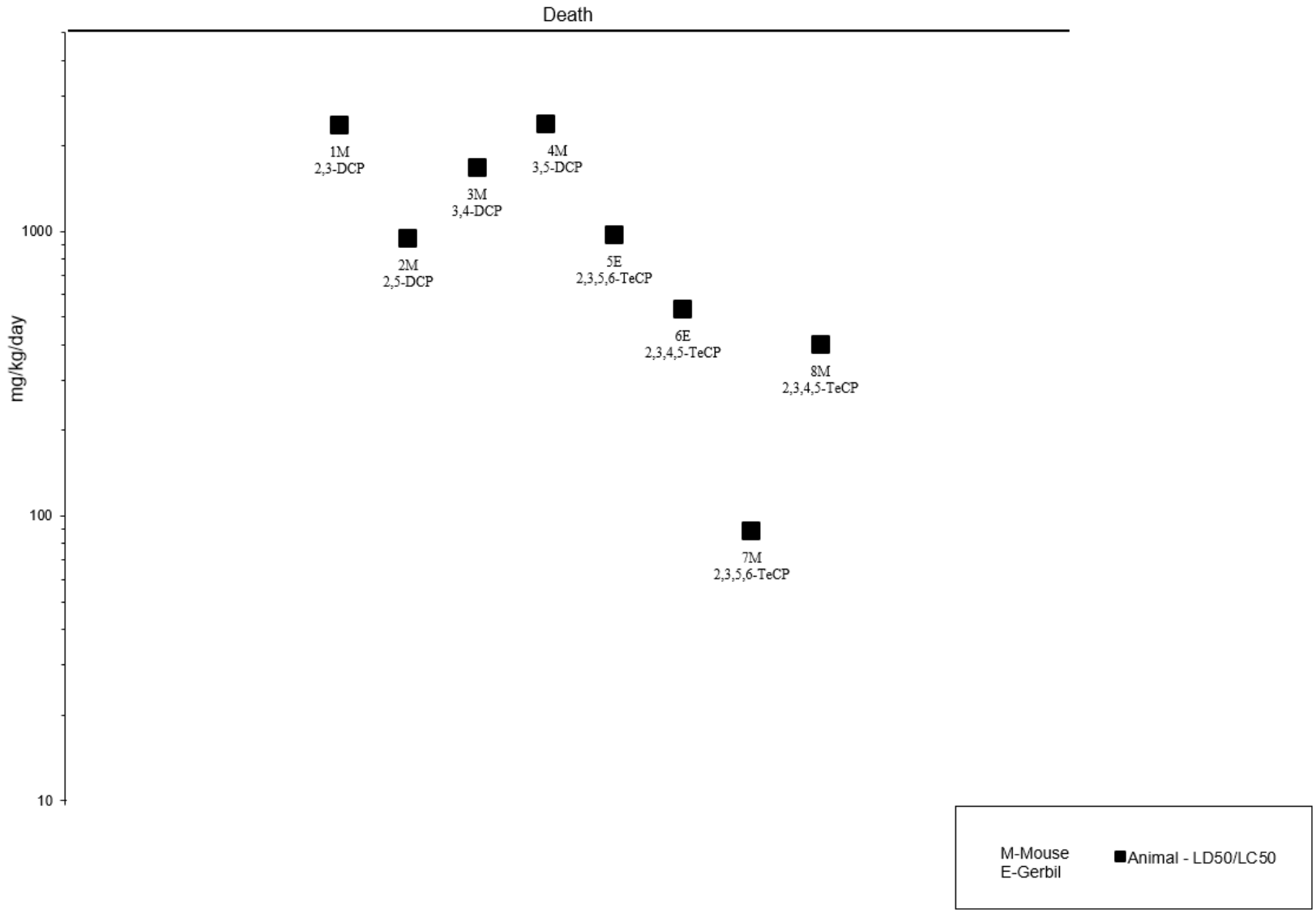


Table 2-9. Levels of Significant Exposure to Chlorophenols – Dermal

Species (strain) No./group	Exposure parameters	Doses (mg/kg)	Parameters monitored	Endpoint	NOAEL (mg/kg)	Less serious LOAEL (mg/kg)	Serious LOAEL (mg/kg)	Effects
ACUTE EXPOSURE								
Rabbit (New Zealand White) 1 M and/or F	24 hours	631, 1,000, 1,580	LE, CS, GN	Death			1,580	2/2 rabbits died
2-Chlorophenol Monsanto 1975								
Mouse (dd) 10–20 M	6 hours	2.5, 5, 10, 25, 50, 100	OW	Immuno	100 M			No effect on ear weight
4-Chlorophenol Dohi et al. 1989								
Rabbit (New Zealand albino) 2 F	24 hours	200, 398	LE, CS, HP, BW	Dermal		200 F		Moderate to marked erythema, edema, and necrosis
2,4-Dichlorophenol Hencke and Lockwood 1978								
Rabbit (New Zealand albino) 2 M	24 hours	250, 500, 1,000, 2,000, 4,000	CS, LE	Death			1,414 M	LD_{50}
				Dermal		250 M		Moderate to marked erythema, slight to marked edema and necrosis
				Neuro		250 M		Lethargy
2,4-Dichlorophenol Carreon et al. 1980b								
Rat (Sprague-Dawley) 10 M, 10 F	24 hours	2,000	LE	Death			2,000 M	1/10 died
2,3,4,5-Tetrachlorophenol Shen et al. 1983								
Rat (Swiss-Webster)	24 hours	485 (M); 565 (F)	LE	Death			485 M	LD_{50}
2,3,4,6-Tetrachlorophenol Shen et al. 1983								

Table 2-9. Levels of Significant Exposure to Chlorophenols – Dermal

Species (strain) No./group	Exposure parameters	Doses (mg/kg)	Parameters monitored	Endpoint	NOAEL (mg/kg)	Less serious LOAEL (mg/kg)	Serious LOAEL (mg/kg)	Effects
Rat (Sprague-Dawley) 10 M, 10 F	24 hours	2,000	LE	Death			2,000 F	2/10 died
2,3,5,6-Tetrachlorophenol								
Shen et al. 1983								

BW = body weight; CS = clinical signs; (F) = feed; F= female(s); GN = gross necropsy; HP = histopathology; LD_{50} = dose producing 50% death; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Neuro = neurotoxicity; NOAEL = no-observed-adverse-effect level; OW = organ weight

2.2 DEATH

Mortality studies of workers at phenoxy herbicide factories where exposure to 2,4,5-TCP, 2,4,6-TCP, and/or 2,4-DCP occurred have not shown increased mortality from any cause (Coggon et al. 1991; Kogevinas et al. 1997; Ott et al. 1987). Occupational studies that focus on cancer-related deaths are discussed in Section 2.19. Case reports of mortalities among workers exposed to 2,4-DCP are discussed below in the section for that chlorophenol.

Acute oral LD_{50} values from animal studies are compared across species, sex, vehicle, and compound in Table 2-10. The data indicate little to no differences in oral LD_{50} values between sexes or species. Vehicle was an important determinant of LD_{50} values for 2,3,4,6- and 2,3,5,6-TeCP, with lower values obtained when these tetrachlorophenols were administered in ethanol compared with propylene glycol. For 2,3,4,5-TeCP, vehicle did not appear to influence LD_{50}. Among compounds tested under the same conditions, there were no marked differences in potency, with the exception of the greater potency (lower LD_{50} values) of 2,3,4,6- and 2,3,5,6-TeCP compared with 2,3,4,5-TeCP when administered in ethanol to mice.

Table 2-10. Comparisons Among Oral LD_{50} Values for Chlorophenols

	Oral LD_{50} (mg/kg)									
Species	Rats		Mice							Gerbils
Sex	NS	Male	Male			Female				Female
Vehicle Compound	Olive oil[a]	Corn oil[b]	diH_20[c]	Corn oil[c]	40% Ethanol[d]	diH_20[c]	Corn oil[c]	40% Ethanol[d]	Propylene glycol[d]	Propylene glycol[d]
2-CP	670	–	347	–	–	345	–	–	–	–
4-CP	670	–	–	1,373	–	–	1,422; 1,640[e]	–	–	–
2,3-DCP	–	–	–	2,585	–	–	2,376	–	–	–
2,4-DCP	–	–	–	1,276	–	–	1,352	–	–	–
2,5-DCP	–	–	–	1,600	–	–	946	–	–	–
3,4-DCP	–	–	–	1,685	–	–	2,046	–	–	–
3,5-DCP	–	–	–	2,643	–	–	2,389	–	–	–
2,4,5-TCP	–	2,960	–	–	–	–	–	–	–	–
2,3,4,5-TeCP	–	–	–	–	572	–	–	400	677	533

Table 2-10. Comparisons Among Oral LD_{50} Values for Chlorophenols

	Oral LD_{50} (mg/kg)									
Species	Rats		Mice							Gerbils
Sex	NS	Male	Male			Female				Female
Vehicle Compound	Olive oil[a]	Corn oil[b]	diH_20[c]	Corn oil[c]	40% Ethanol[d]	diH_20[c]	Corn oil[c]	40% Ethanol[d]	Propylene glycol[d]	Propylene glycol[d]
2,3,4,6-TeCP	–	–	–	–	163	–	–	131	735	698
2,3,5,6-TeCP	–	–	–	–	89	–	–	109	543	979

[a]Deichmann and Mergard 1948
[b]McCollister et al. 1961
[c]Borzelleca et al. 1985b
[d]Ahlborg and Larsson 1978
[e]Shi et al. 2013

CP = chlorophenol; DCP = dichlorophenol; TCP = trichlorophenol; TeCP = tetrachlorophenol

2-CP. In two studies with limited experimental details, nose-only exposure of male and female Wistar rats to 2-CP for 4 hours to a concentration of 908 ppm (Rhone-Poulenc 1991) and whole-body exposure of Sprague-Dawley rats to 2-CP for 6 hours at 620 ppm (Monsanto 1975) did not result in any deaths. In a dose-range-finding study of neonatal Sprague-Dawley rats (postnatal day [PND] 4) given 2-CP by gavage, all animals given 500 mg/kg/day died by the 9th day of exposure, while all survived doses up to 300 mg/kg/day for 18 days in the main study (Hasegawa et al. 2005). When male and female ICR mice were administered daily gavage doses of 35, 69, or 175 mg/kg/day 2-CP in corn oil for 14 days, no exposure-related deaths occurred at the two lower treatment levels, while all mice exposed at 175 mg/kg/day died (Borzelleca et al. 1985a).

Results of a contract laboratory study indicate that the dermal LD_{50} of 2-CP in rabbits is between 1,000 and 1,580 mg/kg (Monsanto 1975). Antemortem observations included increasing weakness, tremors, collapse, and coma. Gross necropsy in the rabbit studies indicated hemorrhage in the lungs, liver discoloration, gastrointestinal inflammation, darkened spleens and kidneys, and enlarged gall bladders. The study data do not clearly indicate whether mortality resulted from any of these effects. Conclusions from this study are limited by small test groups and/or the lack of information regarding experimental methodology.

4-CP. Daily gavage doses of 1,000 mg/kg/day were lethal to 6/12 male and 5/12 female Crl:CD (SD) rats within 12 days of the first dose in a 42–53-day reproductive/developmental toxicity screening study; no deaths occurred at any time at doses up to 200 mg/kg/day (BSRC 2011). Based on clinical signs observed

prior to death, the mortalities were attributed to irritant and central nervous system effects (BSRC 2011). All male and three of four female Sprague-Dawley rats given 500 mg/kg/day 4-CP by gavage beginning on PND 4 died (timing of deaths not reported), while there were no deaths at 300 mg/kg/day in the main study (18 days of exposure, PNDs 4 –21) (Hasegawa et al. 2005).

The acute oral LD_{50} for 4-CP in female ICR mice observed for 7 days after dosing was 1,640 mg/kg/day (Shi et al. 2013). The authors noted that the mice expired within 3 hours of dosing at the highest of the three tested doses (1,575 mg/kg).

2,3-DCP. Borzelleca et al. (1985b) reported acute oral LD_{50} values of 2,585 and 2,376 mg/kg for male and female mice, respectively, given 2,3-DCP as a single dose in corn oil.

2,4-DCP. Four fatalities were reported among chemical workers following acute accidental exposures to 2,4-DCP (CDC 2000). In all cases, the predominant exposure route was dermal, but some effects in lungs and stomach were noted to have been caused by inhalation. A 29-year-old male chemical plant worker lost consciousness almost immediately and died 1 hour after being sprayed with 2,4-DCP on his forearms, right knee, right thigh, and face. CDC (2000) did not report the volume of fluid, concentration of 2,4-DCP in the fluid, or duration of skin contact with the fluid. Pulmonary edema and chemical burns of exposed skin surfaces were the only findings during autopsy. 2,4-DCP levels detected in this patient's blood and urine samples were 13.1 and 6.2 mg/L, respectively. The cause of death was reported as "acute 2,4-dichlorophenol intoxication." A 45-year-old male chemical worker died after being sprayed with steam containing 2,4-DCP (volume and/or concentration not reported). Prior to death, the worker experienced loss of consciousness and convulsions. The time elapsed from exposure to death was not reported. Thermal burns from steam exposure were observed on the skin, mouth, and upper airway, and chemical burns were also observed on the skin. Postmortem findings included pulmonary and laryngeal congestion, alveolar hemorrhage, and hepatocellular fatty change. 2,4-DCP concentrations in biological fluids were not reported. The cause of death was reported as "acute steam and dichlorophenol exposure." A 33-year-old chemical worker died approximately 90 minutes after he was splashed over 60–65% of his body with a solution containing 51% 2,4-DCP. Prior to death, the worker experienced loss of consciousness and convulsions. The autopsy revealed significant damage to the lungs with hemorrhagic fluid in both lungs and in the stomach, as well as intense congestion and petechial hemorrhages in the brain (CDC 2000). Finally, a 64-year-old chemical worker died 20 minutes after 2,4-DCP was splashed on his head and neck (volume and/or concentration not reported). No additional information was reported.

A worker who splattered pure 2,4-DCP on portions of his right arm and leg while disposing of industrial waste collapsed and experienced a seizure within 20 minutes of the accident and died shortly thereafter (CDC 2000; Kintz et al. 1992). Postmortem examination revealed blood and urine 2,4-DCP concentrations of 24.3 and 5.3 mg/L, respectively; concentrations in bile and stomach were 18.7 and 1.2 mg/L, respectively. The identity of 2,4-DCP was confirmed by mass spectrometry, and a screen for other drugs including ethanol, organic solvents, tranquilizers, and drugs of abuse was negative.

When treated on gestation days (GDs) 6–15 with gavage doses of 2,4-DCP in corn oil (750 mg/kg/day), 4 of 34 pregnant Fischer-344 rats died (Rodwell et al. 1989), while all nonpregnant rats treated with 2,000 mg/kg/day in the diet for 14 days survived (NTP 1989). Although pregnant rats may be more susceptible, the difference in effect may also be a result of differences in the rate of exposure between gavage and dietary dosing. All rats and mice exposed to 2,4-DCP in the diet for 13 weeks at doses of 2,000 or 2,600 mg/kg/day survived (NTP 1989). However, all mice died when exposed to 5,200 mg/kg/day for 3 weeks (NTP 1989). In a 2-year study, decreased survival was not observed in rats fed 2,4-DCP in the diet at doses up to 440 mg/kg/day or in mice fed 2,4-DCP in the diet at doses up to 1,300 mg/kg/day for 103 weeks (NTP 1989).

A dermal LD_{50} of 1,415 mg/kg has been reported for male rabbits exposed to 2,4-DCP for 24 hours (Carreon et al. 1980b). Because there were only two rabbits per dose group, the 95% confidence interval (CI) on this value is very large (236–8,455 mg/kg).

2,5-DCP. Oral LD_{50} values of 1,600 and 946 mg/kg were reported for male and female mice, respectively, administered single doses of 2,5-DCP in corn oil (Borzelleca et al. 1985b).

3,4-DCP. In mice given a single dose of 3,4-DCP in corn oil, acute LD_{50} values in males and females were 1,685 and 2,046 mg/kg, respectively (Borzelleca et al. 1985b).

3,5-DCP. Acute oral LD_{50} values of 2,643 and 2,389 mg/kg were reported for male and female mice, respectively, given 3,5-DCP in corn oil as a single dose (Borzelleca et al. 1985b).

2,4,5-TCP. No deaths were observed among rats treated by gavage (18 doses in olive oil) or in the diet with 2,4,5-TCP at doses up to 1,000 mg/kg/day for 90 days (McCollister et al. 1961). In addition, no

deaths were observed in rabbits treated with 20 gavage doses of 500 mg/kg/day 2,4,5-TCP over 28 days (McCollister et al. 1961).

2,4,6-TCP. Deaths were observed during the first 4 weeks of treatment among female (3/40) and male rats (8/25) exposed to 2,4,6-TCP in corn oil by gavage for 11 weeks at 1,000 mg/kg/day, but not at 500 mg/kg/day (Blackburn et al. 1986). The females were treated 2 weeks prior to pregnancy and then throughout gestation. No deaths were observed in rats treated by gavage with 2,4,6-TCP in corn oil at 720 mg/kg/day for 90 days (Bercz et al. 1990). In a 7-week dietary study, 1 of 5 rats died at 1,075 mg/kg/day and 4 of 10 mice died at 4,095 mg/kg/day, with no deaths observed at 735 mg/kg/day among rats or at 2,795 mg/kg/day among mice (NCI 1979). In a chronic study, no increased mortality trend was observed in rats or mice treated with 2,4,6-TCP in the diet at concentrations up to 500 mg/kg/day for 106–107 weeks for rats and 1,356 mg/kg/day for 105 weeks for mice (NCI 1979).

2,3,4,5-TeCP. As shown in Table 2-10, acute oral lethality studies of 2,3,4,5-TeCP resulted in a narrow range of LD_{50} estimates between 400 and 677 mg/kg in male and female mice and female gerbils (Ahlborg and Larsson 1978). Vehicle did not appear to significantly influence the oral lethality of 2,3,4,5-TeCP, in contrast to other tetrachlorophenols: the LD_{50} values in female mice exposed to 2,3,4,5-TeCP were 400 mg/kg when administered in 40% ethanol and 677 mg/kg when administered in propylene glycol (Ahlborg and Larsson 1978).

Unoccluded dermal application of 2,000 mg/kg 2,3,4,5-TeCP resulted in 1 out of 20 deaths in Sprague-Dawley rats (Shen et al. 1983). Clinical signs preceding death included initial hyperactivity followed by hypoactivity, neuromuscular weakness, and convulsions (Shen et al. 1983).

2,3,4,6-TeCP. Oral LD_{50} values for 2,3,4,6-TeCP appear to depend on the vehicle in which it is administered. When administered in ethanol, LD_{50} values of 163 and 131 mg/kg were obtained in male and female mice, respectively (Ahlborg and Larsson 1978). In contrast, when administered in propylene glycol, the LD_{50} values were 735 mg/kg in female mice and 698 mg/kg in female gerbils (Ahlborg and Larsson 1978). No deaths were observed in rats treated by gavage with 200 mg/kg/day 2,3,4,6-TeCP in olive oil during gestation (EPA 1987a, 1987b) or for 90 days (EPA 1986).

In Sprague-Dawley rats, dermal LD_{50} values for commercial tetrachlorophenol, consisting primarily of the 2,3,4,6- isomer (at least 90%), were 485 mg/kg in males and 565 mg/kg in females (Shen et al. 1983).

Prior to death, the rats exhibited initial hyperactivity followed by hypoactivity, neuromuscular weakness, and convulsions.

2,3,5,6-TeCP. In Sprague-Dawley rats exposed by unoccluded dermal application of 2,000 mg/kg 2,3,5,6-TeCP, 2 out of 20 animals died after exhibiting hyperactivity followed by hypoactivity, neuromuscular weakness, and convulsions (Shen et al. 1983).

2.3 BODY WEIGHT

No studies were located regarding body weight effects in humans following exposure to any of the chlorophenols discussed in this profile. In animals, acute-duration oral exposures resulted in adverse effects on body weight at doses as low as 69 mg/kg/day 2-CP in mice (Borzelleca et al. 1985a) and 1,000 mg/kg/day 2,4-DCP in rats (NTP 1989). Higher doses of 2,4-DCP (≥2,000 mg/kg) resulted in serious body weight decrements (more than 25% compared with controls) in both rats and mice (NTP 1989). After intermediate-duration oral exposure, biologically significant decreases in body weight were noted in rats after exposure to ≥100 mg/kg/day 2,3,4,6-TeCP (Dodd et al. 2012; EPA 1986), ≥735 mg/kg/day 2,4,6-TCP (Blackburn et al. 1986; NCI 1979), or 1,000 mg/kg/day 2,4-DCP (NTP 1989), and in mice exposed to 2,600 mg/kg/day 2,4-DCP (NTP 1989). In the few available chronic oral studies in animals, doses of ≥250 mg/kg 2,4-DCP or 2,4,6-TCP in rats resulted in body weight decreases of at least 10% (NCI 1979; NTP 1989). In mice, body weight decreases of up to 19% compared with controls were seen after chronic oral exposure to 820 mg/kg/day 2,4-DCP (NTP 1989).

2-CP. No changes in body weight were observed during the 15-day observation period after rats were exposed (nose only) to 2-CP at 908 ppm for 4 hours in an acute lethality study with no controls (Rhone-Poulenc 1991). In a 14-day study, both sexes of mice receiving 69 mg/kg/day 2-CP had body weight decrements of unspecified magnitude (Borzelleca et al. 1985a); the NOAEL was 35 mg/kg/day. In Sprague-Dawley rats administered 2-CP by gavage at doses up to 257 mg/kg/day for 10 days, no significant effect on body weight was observed (Daniel et al. 1993). Gavage doses up to 1,000 mg/kg/day 2-CP administered to young (5–6 weeks old) Sprague-Dawley rats for 28 days did not result in body weight changes (Hasegawa et al. 2005).

When Sprague-Dawley rats received 2-CP by gavage for 90 days, doses of 150 mg/kg/day resulted in modestly increased body weights at the end of the experiment (11 and 7% higher than controls in males and females, respectively) (Daniel et al. 1993). No effects on body weight were observed in rats treated

with 2-CP in drinking water at doses of 50 mg/kg/day during gestation and lactation (at doses up to 76 mg/kg/day) or up to 7 months of age (at doses up to 62 mg/kg/day) (Exon and Koller 1982, 1983b).

4-CP. Single-day oral exposure of pregnant Sprague-Dawley rats to 1,000 mg/kg 4-CP resulted in significant body weight loss (Kavlock 1990). By 72 hours after dosing, the body weight difference was no longer statistically significantly different from controls. Doses ≤667 mg/kg/day did not inhibit body weight gain (Kavlock 1990). Gavage doses up to 500 mg/kg/day 4-CP administered to young (5–6 weeks old) Sprague-Dawley rats for 28 days did not result in body weight changes (Hasegawa et al. 2005). At 1,000 mg/kg/day for 42–53 days, 4-CP induced significant reductions in body weight and food consumption; this dose was also lethal to about half of exposed rats (BSRC 2011).

2,4-DCP. Body weights of pregnant animals treated on GDs 6–15 were reduced at 375 mg/kg/day, but not at 200 mg/kg/day (Rodwell et al. 1989). No body weight effects were observed in BALB/c mice receiving 2,4-DCP in drinking water (~260 mg/kg/day) for 14 days (Aydin et al. 2009). Studies with rats and mice fed diets containing 2,4-DCP for acute, intermediate, and chronic durations revealed dose-related decreases in food intake and body weight that were attributed to poor palatability of the treated diets (NTP 1989). Body weights were not affected in mice treated with 2,4-DCP in the diet at doses up to 230 mg/kg/day (Kobayashi et al. 1972) or in drinking water at doses up to 491 mg/kg/day (in females) or 383 mg/kg/day (in males) (Borzelleca et al. 1985a, 1985c). To improve palatability in drinking water, Borzelleca et al. (1985a, 1985c) added a 1:9 emulphor:water solution (modified vegetable oil). Body weights were not affected in Sprague-Dawley rats exposed to 2,4-DCP from conception through weaning and for an additional 15 weeks in drinking water at doses up to 44 mg/kg/day (Exon and Koller 1985; Exon et al. 1984).

Body weight effects were observed in a 2-generation reproductive toxicity study in Wistar-Hanover rats (Aoyama et al. 2005). Groups of 24 rats/sex/group were administered a diet containing 2,4-DCP at 0, 500, 2,000 or 8,000 ppm, which corresponded to 0, 33.4, 134, or 543 mg/kg/day for males and 0, 49.1, 194, or 768 mg/kg/day for females. Feed aversion was apparent since body weight gain and feed consumption were significantly decreased in mid-dose P generation females at the end of the pre-mating, and during the gestational periods, and in high-dose P and F1 generation males and females throughout exposure (Aoyama et al. 2005).

2,4,5-TCP. Treatment of rats by gavage with 2,4,5-TCP for 18 or 24 days at 1,000 mg/kg/day had no effect on body weight (McCollister et al. 1961). In contrast, treatment with 2,4,5-TCP in the diet at

1,000 mg/kg/day for 90 days resulted in a 24% decrease in body weight gain in female rats, but not in male rats (McCollister et al. 1961). No effects on food intake were measured.

2,4,6-TCP. Treatment of rats with 2,4,6-TCP by gavage at 1,000 mg/kg/day for 2 weeks before mating and throughout gestation resulted in reduced body weights through GD 14 (Blackburn et al. 1986). Body weights on GD 21 were not significantly different from those of the controls. No effect on body weight was observed in rats treated by gavage with 2,4,6-TCP at 1,000 mg/kg/day for 90 days (Bercz et al. 1990) or 11 weeks (Blackburn et al. 1986), suggesting that pregnant animals may be more sensitive to effects on body weight following treatment with 2,4,6-TCP. No effect on body weight was observed in rats treated with 2,4,6-TCP in drinking water at 44 mg/kg/day from conception through weaning and for an additional 10–15 weeks (Exon and Koller 1985). Body weights were significantly reduced in rats treated with 2,4,6-TCP in the diet for 7 weeks at 735 mg/kg/day, but not at 500 or 250 mg/kg/day, for 105 weeks (NCI 1979). Body weights were also significantly decreased in mice fed 2,600 mg/kg/day 2,4,6-TCP in the diet for 7 weeks and at 658 mg/kg/day for 105 weeks (NCI 1979). No effects on body weight were observed in mice fed 1,300 mg/kg/day 2,4,6-TCP for 7 weeks (NCI 1979). Food intake data were not provided in the NCI (1979) study. The fact that 2,4,6-TCP affected body weight following dietary intake but had little effect at similar doses following gavage treatment suggests that 2,4,6-TCP may have caused the food to be less palatable and reduced food intake in mice at the concentrations used in the NCI (1979) study. Therefore, decreased body weight may be an effect of decreased food intake rather than an effect of 2,4,6-TCP treatment.

2,3,4,6-TeCP. Acute-duration exposure to 2,3,4,6-TeCP at doses up to 200 mg/kg/day for 5 days did not result in any body weight changes (Dodd et al. 2012). In the same study, treatment for 2 or 4 weeks at 200 mg/kg/day similarly had no effect on body weight (Dodd et al. 2012). Body weight was significantly decreased in rats treated by gavage with 2,3,4,6-TeCP at 100 mg/kg/day for 90 days (Dodd et al. 2012; EPA 1986), but not 100 mg/kg/day for 55 days (Hattula et al. 1981).

2.4 RESPIRATORY

Human data on respiratory effects of chlorophenols are limited by confounding by co-exposures, poor exposure characterization, and small numbers of exposed subjects. No repeated-exposure animal studies of chlorophenol exposure by inhalation were located. In oral animal studies, only one (NTP 1989) reported respiratory system effects. Nasal lesions were seen in male rats fed 210 mg/kg/day 2,4-DCP in

feed for 103 weeks, but not in female rats or in mice fed as much as 1,300 mg/kg/day. It is possible that the lesions occurred from aspiration while eating.

When compared to 260 unexposed referents, 281 workers involved in the production of sodium trichlorophenol (2,4,5-TCP) and its derivatives for 18 years had no increased incidence of chronic bronchitis, chronic obstructive pulmonary disease, or altered measures of pulmonary function (Calvert et al. 1991). Occupational exposure of seven workers to an unspecified trichlorophenol isomer, in addition to other chemicals, by chronic inhalation for 2–10 years was associated with adverse upper airway and chest symptoms (cough, chronic bronchitis, chest wheezing), altered pulmonary function (reduced expiratory flow rate of the lung, increased closing volume of the lung, increased elastic recoil pressure of the lung), and pulmonary lesions (interstitial densities) (Alexandersson and Hedenstierna 1982). The air exposure concentrations were characterized as 0.003 mg/L (0.02 ppm) trichlorophenol or less, with potential for considerable variability. The study was also limited by the very small number of subjects (seven), which included three smokers. It is, therefore, not possible to determine whether the exposure to trichlorophenol alone induced the reported respiratory effects or whether smoking was a contributing factor.

Lumber mill workers (40 exposed and 40 controls) exposed to a mixture of tetrachlorophenols (specific isomers not stated) and pentachlorophenol reported upper respiratory tract irritation more frequently than unexposed workers (Kleinman et al. 1986). Tetrachlorophenol air concentrations ranged from 0.8 to 12.2 $\mu g/m^3$, and pentachlorophenol concentrations were below the limit of detection (0.5 $\mu g/m^3$).

In a cross-sectional study of 2,125 children and adults participating in NHANES (2005–2006) (Jerschow et al. 2014), urinary levels of 2,5-DCP were associated with increased odds of all self-reported measures of asthma (told by doctor; prescribed medication for wheezing; missed work due to wheeze; and wheezing during exercise; odds ratios (ORs) for 3rd tertile compared to 1st tertile ranged from 2.2 to 10.0, and p-value for trend <0.05 for all measures) among atopic wheezers (n=156). Urinary 2,4-DCP was associated only with an increased odds of missing work due to wheezing (OR 11.4 for 3rd tertile compared to 1st tertile; p-value for trend <0.01) among atopic wheezers (Jerschow et al. 2014). Among non-atopic wheezers (n=94), none of the asthma metrics were clearly increased with urinary concentrations (Jerschow et al. 2014). In a study of 3,617 adult NHANES (2007–2010) participants (Rooney et al. 2018), no association between prevalence of respiratory diseases (asthma, chronic bronchitis, or emphysema) and urinary 2,4- or 2,5-DCP levels was observed.

2-CP. In a 4-hour inhalation (nose only) exposure to 2-CP, tachypnea was observed in one of five male, but not female, rats at 908 ppm (Rhone-Poulenc 1991). Dark red foci were observed in the lungs (right caudal, median, or left lobe) of male and female rats exposed to 17 (2/5 males, 2/5 females) or 104 ppm (4/5 males, 2/5 females) but were not found at 908 ppm (Rhone-Poulenc 1991). No controls were used in this study. There were no treatment-related changes in lung weights or gross or microscopic pathology findings in the lungs of Sprague-Dawley rats administered 2-CP by gavage at doses up to 257 mg/kg/day for 10 days (Daniel et al. 1993), up to 1,000 mg/kg/day for 28 days (Hasegawa et al. 2005), or up to 150 mg/kg/day for 90 days (Daniel et al. 1993).

4-CP. When young (5–6 weeks old) Sprague-Dawley rats were administered 4-CP at doses up to 500 mg/kg/day by gavage for 28 days, lung weights were not affected, and no treatment-related microscopic pathology findings were noted in the lungs (Hasegawa et al. 2005). BSRC (2011) reported dyspnea and abnormal respiratory noises in male rats given 1,000 mg/kg/day 4-CP by gavage for 42 days; this dose was lethal to 6/12 males.

2,4-DCP. Lung hemorrhaging occurred in rats treated with a single lethal gavage dose of 2,4-DCP (Wil Research Laboratories 1982). Nasal lesions were noted in male but not female rats fed 210 mg/kg/day for 103 weeks; however, no nasal lesions were observed in mice fed as much as 1,300 mg/kg/day for the same duration (NTP 1989). Therefore, this effect may be specific to the male rat or may have been a result of aspiration while eating. Histopathological changes have not been observed in the lungs of rats exposed to 2,4-DCP at doses up to 5,200 mg/kg/day in feed (NTP 1989). In chronic-duration studies, neither rats nor mice exhibited treatment-related microscopic changes in the lungs after exposure to 2,4-DCP in the diet for 103 weeks at doses up to 440 mg/kg/day (rats) or 1,300 mg/kg/day (mice) (NTP 1989).

2,4,5-TCP. No lung weight changes or histopathological changes in the lungs were observed in rats given 2,4,5-TCP by gavage at doses up to 1,000 mg/kg/day for 18 exposures over 24 days, in rabbits given doses up to 500 mg/kg/day for 20 exposures over 28 days, or in rats exposed for 98 days via the diet at concentrations up to 1% in feed (about 1,000 mg/kg/day) (McCollister et al. 1961).

2,4,6-TCP. Histopathological changes were not observed in the lungs of rats or mice following oral administration of 2,4,6-TCP for 5–13 weeks at doses up to 1,000 mg/kg/day (Bercz et al. 1990; Blackburn et al. 1986; NCI 1979) or for 103 weeks at doses up to 500 mg/kg/day (rats) or 1,356 mg/kg/day (mice) (NCI 1979).

2,3,4,6-TeCP. No histopathological changes were observed in the lungs of rats orally exposed to 2,3,4,6-TeCP (doses up to 2,000 mg/kg/day by daily gavage) for 90 days (EPA 1986).

2.5 CARDIOVASCULAR

No adequate studies of cardiovascular effects in humans exposed to chlorophenols by any route were located. A number of cross-sectional studies of chlorophenols in urine and cardiovascular endpoints in humans have been published. However, these studies are of limited utility for hazard identification because exposures are measured at the same time or after the health effect is assessed, and because chlorophenols in urine can occur as a result of metabolism of other compounds such as chlorinated benzenes (see Section 3.3.1 for further information). In available studies of animals exposed orally to chlorophenols, no effects on heart weights or cardiac histopathology were reported.

Electrocardiograms were normal in three individuals who developed chloracne following occupational exposure (inhalation and dermal) to chlorophenols and other compounds during the manufacture of 2,4-DCP and 2,4,5-TCP (Bleiberg et al. 1964).

Available cross-sectional studies have not shown associations between urinary concentrations of 2,4-DCP and obesity, overweight, body mass index (BMI), waist circumference, serum triglycerides or cholesterol, or blood pressure in children or adults (Parastar et al. 2018; Shiue 2014; Shiue and Hristova 2014). Similarly, no association between urinary 2,4-DCP levels and cardiovascular disease (coronary heart disease, heart attack, chronic heart failure, or stroke) prevalence was observed in a study of 3,617 adult NHANES (2007–2010) participants (Rooney et al. 2018). In a study in Iran, urinary levels of 2,5-DCP were associated with higher BMI z-score and waist circumference in 6–18-year-old children and adolescents and with lower systolic blood pressure in 6–11-year-old children (Parastar et al. 2018). However, urinary 2,5-DCP was not associated with high blood pressure in studies of adult NHANES participants (Shiue 2014; Shiue and Hristova 2014). Rooney et al. (2018) observed positive associations between urinary 2,5-DCP concentrations and coronary heart disease, but not with chronic heart failure or stroke in adult NHANES (2007–2010) participants. Parastar et al. (2018) observed a positive association between urinary 2,4,5-TCP and waist circumference among 12–18-year-old adolescents in Iran, and inverse associations between 2,4,5-TCP and high-density lipoprotein (HDL) cholesterol in both 6–11- and 12–18-year-old children and adolescents.

2-CP. In Sprague-Dawley rats administered up to 257 mg/kg/day 2-CP by gavage for 10 days, no treatment-related changes in heart weight or histopathology were observed (Daniel et al. 1993). Heart weights were not affected, and no microscopic heart changes were seen in Sprague-Dawley rats after 28 days of exposure (beginning at 5–6 weeks of age) to gavage doses up to 1,000 mg/kg/day 2-CP (Hasegawa et al. 2005). In a 90-day study by Daniel et al. (1993), neither cardiac weight nor microscopic findings in the heart were affected in Sprague-Dawley rats receiving doses up to 150 mg/kg/day 2-CP.

4-CP. When young Sprague-Dawley rats were given up to 500 mg/kg/day 4-CP by gavage for 28 days of exposure (beginning at 5–6 weeks of age), neither heart weight nor histology was affected by exposure (Hasegawa et al. 2005).

2,4-DCP. Heart weights of mice fed doses of up to 230 mg/kg/day 2,4-DCP for 6 months were not changed (Kobayashi et al. 1972). Studies of intermediate and chronic durations of 2,4-DCP-fed rats (fed 2,000 and 440 mg/kg/day, respectively) and mice (fed 2,600 and 1,300 mg/kg/day, respectively) showed no effect on histopathological examination of the heart (NTP 1989).

2,4,5-TCP. No heart weight nor histologic changes were observed in rats treated by gavage with 1,000 mg/kg/day of 2,4,5-TCP for 18 (out of 24) days, nor were histological changes observed in the hearts of rats treated with up to 1,000 mg/kg/day 2,4,5-TCP in the diet for 98 days (McCollister et al. 1961).

2,4,6-TCP. Treatment of rats orally administered doses as high as 1,000 mg/kg/day 2,4,6-TCP showed no change in heart weight over an intermediate (10 or 13 weeks) exposure period (Bercz et al. 1990; Blackburn et al. 1986). No treatment-related lesions were evident upon histopathologic examination of the hearts of rats and mice exposed to doses of 2,4,6-TCP as high as 720 mg/kg/day for 90 days (Bercz et al. 1990) and 1,356 mg/kg/day for 105 weeks (NCI 1979).

2,3,4,6-TeCP. No changes in heart weight or histology were observed in rats treated with 2,3,4,6-TeCP for 90 days (EPA 1986).

2.6 GASTROINTESTINAL

The self-reported prevalence of gastrointestinal disease was not increased among 281 TCP production workers with elevated serum markers of exposure (TCDD) (Calvert et al. 1992). However, the long lag time (at least 15 years) between exposure and examination of gastrointestinal symptoms compared with the rapid elimination of chlorophenols may invalidate the results. Few animal studies have indicated gastrointestinal effects after oral exposure to chlorophenols; when effects were observed, they typically followed gavage administration of high doses (≥1,000 mg/kg). The one exception is 2,3,4,6-TeCP; necrosis in the intestines was reported in Wistar rats given ≥100 mg/kg/day 2,3,4,6-TeCP by gavage for 55 days (Hattula et al. 1981).

2-CP. Sprague-Dawley rats exposed by gavage for 10 days to 2-CP doses up to 257 mg/kg/day or for 90 days to doses up to 150 mg/kg/day exhibited no treatment-related effects on gastrointestinal tract histology (Daniel et al. 1993).

4-CP. Histopathology findings consisting of squamous epithelial hyperplasia in the forestomach and erosion or ulcers in the esophagus and forestomach were observed in rats given 1,000 mg/kg/day 4-CP by gavage for up to 53 days; mortalities at this dose were partially attributed to these gastrointestinal effects (BSRC 2011). There were no histopathology examinations in the gastrointestinal tracts of animals receiving lower doses, so a NOAEL could not be identified.

2,4-DCP. Mild catarrhal enteritis was observed in female Sprague-Dawley albino rats given a single gavage dose of 316–5,000 mg/kg 2,4-DCP in corn oil and sacrificed 24 hours later (Hencke and Lockwood 1978). In another study, gross necropsy revealed reddened hindstomach and intestines in Fischer-344 rats given a single gavage dose of 2,400 mg/kg/day 2,4-DCP in corn oil (Wil Research Laboratories 1982). Following 2,4-DCP exposure in feed, no significant histopathological changes were observed in the gastrointestinal tracts of Fischer-344 rats fed 2,000 mg/kg/day, mice fed 2,600 mg/kg/day for 13 weeks, rats fed 440 mg/kg/day, or mice fed 1,300 mg/kg/day for 103 weeks (NTP 1989).

Diarrhea was observed in one of two female rabbits the day after a dermal exposure to a single dose of 398 mg/kg 2,4-DCP (Hencke and Lockwood 1978). This limited study suggests that either dermally applied 2,4-DCP, or the stress of being exposed to a skin irritant, can result in gastrointestinal effects in rabbits.

2,4,6-TCP. McCollister et al. (1961) reported diarrhea in rats given single gavage doses (≥1,000 mg/kg) of 2,4,5-TCP in an acute lethality study. These authors also reported wet abdominal areas among rats receiving 2,4,5-TCP at doses ≥300 mg/kg/day in the diet for 3 months, and suggested that the rats probably had diarrhea (McCollister et al. 1961).

2,4,6-TCP. In a 90-day study, no significant histopathological changes were observed in the gastrointestinal tracts of rats treated by gavage with 2,4,6-TCP at 720 mg/kg/day (Bercz et al. 1990). Histopathologic examination of the stomach and intestines of rats and mice exposed for 2 years to doses as high as 500 and 1,356 mg/kg/day 2,4,6-TCP, respectively, revealed no treatment-related lesions (NCI 1979).

2,3,4,6-TeCP. Wistar rats administered a single gavage dose of 632 mg/kg 2,3,4,6-TeCP had eosinophilic granulocyte infiltration in the stomach, mucosal hyperemia of the small intestine, and severe necrosis of the large intestine (Hattula et al. 1981). At doses of 432 and 518 mg/kg, mild necrosis was observed in the large intestines of 1/10 rats (each), with 70% of animals showing necrosis at 622 mg/kg; no effects on the large intestine were observed at 410 mg/kg. Focal necrosis of the small intestines was observed in Wistar rats treated by gavage for 55 days with 100 mg/kg/day2,3,4,6-TeCP, but not 10 mg/kg/day (Hattula et al. 1981). In contrast, no histopathological changes were observed in the gastrointestinal tracts of Sprague-Dawley rats treated with 2,3,4,6-TeCP at 200 mg/kg/day for 90 days (EPA 1986). 2,3,4,6-TeCP was administered in olive oil in both the Hattula et al. (1981) (concentrations not reported) and EPA (1986) studies (maximum concentration of 20 mg/mL). Because olive oil was used as a vehicle for both studies, the difference in gastrointestinal tract effects may be due to dosing solution concentrations or rodent strain.

2.7 HEMATOLOGICAL

Human data are inadequate to assess the hematological effects of chlorophenols, while animal studies do not indicate that the hematopoietic system is a sensitive target of oral exposure to chlorophenols. Clinical assessment of two patients occupationally exposed during the manufacture of 2,4-DCP- and 2,4,5-TCP-based herbicides showed hematology and blood chemistry parameters (blood counts, bleeding and clotting time, serum bilirubin, blood urea nitrogen [BUN], and others) to be within normal ranges (Bleiberg et al. 1964).

2-CP. Groups of mice (12/sex) were administered up to 69 mg/kg/day 2-CP daily by gavage in corn oil for 14 days. No adverse effects on standard hematological parameters, including total and differential white blood cells, red blood cells, platelets, hematocrit, hemoglobin, and coagulation measures were reported relative to unexposed controls (Borzelleca et al. 1985a). In a 10-day study, Daniel et al. (1993) found that doses up to 257 mg/kg/day 2-CP resulted in increased red blood cell count (12%) and hematocrit (28%) in male, but not female, Sprague-Dawley rats. However, the investigators indicated that the values remained within normal ranges for laboratory rats.

No hematologic changes (both erythrocyte and leukocyte parameters were measured) were observed in Sprague-Dawley rats exposed to 2-CP at doses up to 1,000 mg/kg/day for 28 days (Hasegawa et al. 2005). In a subchronic (90 day) study, doses up to 150 mg/kg/day 2-CP administered by gavage resulted in increased red blood cell count and hematocrit in females and increased mean corpuscular volume in males at 150 mg/kg/day (Daniel et al. 1993). Intermediate-duration pre- and postnatal (from conception through weaning at PND 21) exposure to 2-CP in drinking water (up to 73 mg/kg/day 2-CP) did not adversely affect red blood cell count, hematocrit, mean corpuscular volume, white cell count, or hemoglobin concentration (Exon and Koller 1982). However, pre- and postnatal exposure to 62 mg/kg/day 2-CP in drinking water for up to 24 months or until death resulted in increased (>10%) erythrocyte count and hemoglobin concentration and an increase in packed cell volume (Exon and Koller 1985). The investigators speculated that the increase may be secondary to effects on liver enzymes or hematopoietic stem cells and did not consider these effects biologically significant (Exon and Koller 1985).

4-CP. No changes to erythrocyte or leukocyte parameters were observed when Sprague-Dawley rats were given 4-CP by gavage (up to 500 mg/kg/day) for 28 days (Hasegawa et al. 2005).

2,4-DCP. Groups of 12 male and 12 female mice administered up to 638 mg/kg/day 2,4-DCP (by gavage in corn oil vehicle) for 14 days showed no adverse effects on hematological parameters, including total and differential white blood cells, red blood cells, platelets, hematocrit, hemoglobin, and coagulation measures relative to unexposed controls (Borzelleca et al. 1985a). However, when groups of 20 male and 20 female mice were dosed with up to 383 mg/kg/day of 2,4-DCP (male) and 49 mg/kg/day (female) in drinking water (containing 10% Emulphor) for 90 days, the number of white blood cells was increased in the high-dose males (Borzelleca et al. 1985a, 1985c). No changes in red or white blood cell counts were noted in mice exposed to 2,4-DCP at doses up to 230 mg/kg/day for 6 months (Kobayashi et al. 1972). In an NTP 13-week study (NTP 1989), bone marrow atrophy was observed in male rats treated with 2,4-DCP in the diet at 1,000 mg/kg/day and in female rats at 500 mg/kg/day. The atrophy resulted in

depletion of both erythroid and myeloid elements, with no effects observed at 250 mg/kg/day. No hematological effects were noted in mice treated with up to 2,600 mg/kg/day 2,4-DCP in the diet for 13 weeks or in rats or mice treated with 440 or 1,300 mg 2,4-DCP/kg/day, respectively, for 103 weeks (NTP 1989).

Chronic prenatal and postnatal exposure to 44 mg/kg/day 2,4-DCP resulted in increased (>10%) erythrocyte count and hemoglobin concentration, and an increase in packed cell volume (Exon and Koller 1985). As discussed above for 2-CP, the investigators believed these results to be secondary effects that were not biologically significant.

2,4,5-TCP. Treatment of rats with 2,4,5-TCP in the diet at doses up to 1,000 mg/kg/day for 98 days resulted in no changes in hematocrit, hemoglobin, or white blood cell counts (McCollister et al. 1961).

2,4,6-TCP. Administration of up to 720 mg/kg/day 2,4,6-TCP by gavage to rats for 90 days resulted in no adverse effects on erythrocyte count, leukocyte count, corrected leukocyte count, hemoglobin, hematocrit, platelet count, or a differential analysis of leukocytes (Bercz et al. 1990). Rats exposed orally for 7 weeks to 4,600 mg/kg/day 2,4,6-TCP exhibited a “moderate to marked increase” in splenic hematopoiesis (NCI 1979). A high incidence of bone marrow hyperplasia, leukocytosis, and monocytosis occurred in rats chronically exposed to 2,4,6-TCP in their diet at 250 or 500 mg/kg/day (NCI 1979). Further discussion of these hematological effects in rats can be found in Section 2.19. No hematological effects were evident in mice exposed chronically to 2,4,6-TCP in their diet at doses up to 1,300 mg/kg/day (NCI 1979).

2,3,4,6-TeCP. Treatment of rats by gavage with doses of 200 mg/kg/day 2,3,4,6-TeCP for 90 days significantly ($p<0.05$) reduced hemoglobin and hematocrit in both sexes (EPA 1986). Although the effects were statistically significant, the investigators did not consider the effects to be toxicologically significant because the group mean data were within the normal range of reference control data for the laboratory where the study was conducted. In addition, no gross or histopathologic evidence was found to support the decreases in hemoglobin and hematocrit.

2.8 MUSCULOSKELETAL

Wang et al. (2020) conducted a cross-sectional study of bone mineral density (BMD) and urinary dichlorophenols using NHANES data (2005–2010). The participants, all at least 20 years of age, included

2,267 men (mean age 45.8 years); 1,145 premenopausal women (mean age 37.3); and 1,033 postmenopausal women (mean age 62.6). Concentrations of 2,4- and 2,5-DCP in urine were used as measures of exposure. In men, urinary concentrations of 2,4-DCP were associated with lower BMD (regression coefficients [βs] ranged between -7.41 and -8.31 depending on the bone tissue analyzed). In addition, 2,5-DCP concentrations in men were associated with higher prevalence of osteopenia and osteoporosis (OR 1.15; 95% CI 1.03, 1.28). No associations between urinary dichlorophenols and BMD, osteopenia, or osteoporosis were observed in pre- or postmenopausal women. In animals, studies of musculoskeletal endpoints after oral exposure to chlorophenols were limited to muscle and bone histopathology examinations in studies of 2-CP, 2,4-DCP, and 2,3,4,6-TeCP. No treatment-related effects were reported.

2-CP. No microscopic lesions were identified in the skeletal muscle or bones of Sprague-Dawley rats given 2-CP by gavage at doses up to 257 mg/kg/day for 10 days or up to 150 mg/kg/day for 90 days (Daniel et al. 1993).

2,4-DCP. Ninety-day (up to 2,600 mg/kg/day) and 2-year (up to 1,300 mg/kg/day) dietary exposures of rats and mice to 2,4-DCP did not result in any histopathological changes in the muscle or ribs (NTP 1989).

2,3,4,6-TeCP. Single-dose (up to 632 mg/kg) and 55-day (up to 100 mg/kg/day) exposures to 2,3,4,6-TeCP produced no adverse histopathological effects on muscle in Wistar rats (Hattula et al. 1981).

2.9 HEPATIC

The limited available data on the hepatic effects of chlorophenols in exposed humans are potentially confounded by coexposures to other chemicals and alcohol use, rendering them of little utility for hazard identification. The liver is a well-established target of chlorophenol toxicity in laboratory animals exposed orally. Hepatic effects including clinical chemistry changes, increased liver weight, hepatocellular hypertrophy, and necrosis have been observed in rats and mice after acute, intermediate, and/or chronic oral exposure to 2-CP, 4-CP, 2,4-DCP, 2,4,5-TCP, 2,4,6-TCP, and 2,3,4,6-TeCP (Aydin et al. 2009; Bercz et al. 1990; BSRC 2011; Dodd et al. 2012; Exon and Koller 1985; Exon et al. 1984; Hasegawa et al. 2005; Kobayashi et al. 1972; McCollister et al. 1961; NCI 1979; NTP 1989).

Porphyria cutanea tarda (a skin condition caused by markedly decreased uroporphyrinogen decarboxylase activity in the liver) has been reported in workers employed in the manufacture of 2,4-DCP and 2,4,5-TCP (Bleiberg et al. 1964). Exposure to chlorophenols and intermediates was likely through inhalation and dermal contact. In a survey of 29 workers, 11 cases of porphyria were identified, based on urinary porphyrin excretion and 2 were studied in more detail. In the two cases, elevated serum transaminase levels and evidence of liver damage, e.g., regeneration of liver cells and hemofuscin (a brownish-yellow pigment that results from the decomposition of hemoglobin) deposition, were detected from liver biopsies indicating the exposure was related to liver injury. No definitive conclusions regarding the connection between the porphyria or liver injury and exposure to chlorophenols in this group of workers can be made because the workers were exposed to a variety of chlorinated compounds, including a highly volatile chlorinated phenolic ether with six chlorines formed during the manufacturing process. Information on exposure to other liver toxicants, including the chronic ingestion of alcohol, was not obtained.

The results of a cross-sectional study of 281 trichlorophenol exposed production workers (and 260 controls) indicated an increased risk of elevated gamma-glutamyltransferase (GGT) activity in these workers (OR 2.27; CI 1.17–4.39, not adjusted for confounding factors) (Calvert et al. 1992). Statistical evaluation of interactions indicated elevated risk was restricted to workers with a history of alcohol consumption. Risk of increased GGT activity in workers with a history of alcohol consumption correlated with increased exposure (Calvert et al. 1992). Because the effect was seen only in those workers who consumed alcohol, and because the workers were also exposed to other compounds (including TCDD), and other hepatic enzymes were not increased, the utility of this study for hazard identification is limited.

In a cross-sectional study of 3,617 adult NHANES (2007–2010) participants (Rooney et al. 2018), no associations were observed between the prevalence of self-reported nonneoplastic liver conditions (not further specified) and urinary 2,4-DCP or 2,5-DCP levels.

2-CP. In a 10-day study in Sprague-Dawley rats exposed by gavage, no changes in liver weight, serum chemistry, or histology were observed at doses up to 257 mg/kg/day (Daniel et al. 1993). Mice administered doses up to 69 mg/kg/day 2-CP in corn oil by gavage for 14 days exhibited a significant decrease in liver weights in females with no effects on serum aspartate aminotransferase (AST) or alanine aminotransferase (ALT); liver microsomal proteins; cytochrome P-450; cytochrome b5; activities of liver aminopyrine demethylase, aniline hydroxylase, or aryl hydrocarbon hydroxylase; or gross pathology

findings (Borzelleca et al. 1985a). The results of the study were reported qualitatively, and histopathology examinations were not performed, so the toxicological significance of the liver weight decrease is not known.

Sprague-Dawley rats administered 1,000 mg/kg/day 2-CP by gavage for 28 days exhibited increased incidences of slight centrilobular hepatocellular hypertrophy (6/6 males and 5/6 females, compared with 0/6 male and 0/6 female controls) (Hasegawa et al. 2005). There were no microscopic findings in the livers of animals receiving lower doses (up to 500 mg/kg/day) (Hasegawa et al. 2005). An intermediate-duration study conducted by Daniel et al. (1993) showed no effects on liver weight, serum chemistry, or liver histopathology in Sprague-Dawley rats after 90 days of exposure to 2-CP at doses as high as 150 mg/kg/day (Daniel et al. 1993).

4-CP. Seven days after a single gavage dose up to 1,575 mg/kg 4-CP, liver weights of female ICR mice were not different from controls (Shi et al. 2013). In Sprague-Dawley rats, twice daily administration of as little as 0.32 mg/kg 4-CP for 2 weeks (0.64 mg/kg/day) resulted in significant activation of hepatic enzymes including cytochrome P-450, as well as elevated levels of microsomal protein and cytochrome P-450, without altering the liver/body weight ratio (Phornchirasilp et al. 1989b). The magnitude of increase in liver microsomal protein and cytochrome P-450 content over 2 weeks declined at doses >0.64 mg/kg/day. Following additional experiments in which treatment was given 2 times/day, both a 2-week exposure to 2.58 mg/kg/day and a ≥4-week exposure to 0.64 mg/kg/day resulted in morphological changes in hepatic ultrastructure (foamy cytoplasm and the proliferation and clustering of mitochondria and endoplasmic reticulum). The electron microscopic changes were not observed in the livers of rats treated at 1.28 mg/kg/day for 2 weeks. In separate studies, similar treatment doses of 4-CP had no effect on relative liver weights, microsomal zoxazolamine 6-hydroxylase activity, or serum lipids and lipoprotein concentrations, but did increase fasting glucose levels (Phornchirasilp et al. 1989a).

When Sprague-Dawley rats received 4-CP by gavage for 28 days, no liver histopathology findings were seen by light microscopy at doses up to 500 mg/kg/day (Hasegawa et al. 2005). In a reproductive/ developmental toxicity screening study, rats given 1,000 mg/kg/day for 42–53 days exhibited increased incidences of centrilobular hepatocellular hypertrophy (BSRC 2011). Histopathology examinations of the liver were not performed at lower doses in this study, so a NOAEL could not be determined.

In a study examining the role of oxidative stress on hepatic effects of 4-CP, ICR mice were given 0, 1, 10, or 100 mg/kg/day in corn oil by gavage for 28 days, and hepatic levels of superoxide dismutase (SOD),

catalase (CAT), and malondialdehyde (MDA) (all normalized to protein levels in the liver) were measured (Shi et al. 2013). A significant increase in MDA was observed at the highest dose, suggesting that 4-CP may induce oxidative stress in the livers of mice. No apical endpoints were evaluated in this study.

2,4-DCP. In a 2-week study focused on male reproductive toxicity, significantly increased AST, ALT, and lactate dehydrogenase (LDH) were observed in BALB/c mice given 1,000 ppm 2,4-DCP in drinking water (~260 mg/kg/day) (Aydin et al. 2009). No changes in liver weight were reported. No other hepatic parameters were evaluated in this study. When guinea pigs were administered 40 mg/kg 2,4-DCP orally 3 times/week for 2 weeks, lipid peroxidation was increased in the liver (Clerhata et al. 1996). A high intake of ascorbic acid (50 mg/animal/day) significantly decreased lipid peroxidation in the liver in comparison to guinea pigs with low ascorbic acid intake (2 mg/kg/day). 2,4-DCP accumulation was also decreased in the liver of animals with high ascorbic acid intake.

Sprague-Dawley rats dosed at 44 mg/kg/day of 2,4-DCP *in utero* and through lactation (via maternal exposure in the drinking water) and postweaning in the drinking water for about 15 weeks exhibited increased absolute liver weights (19% higher than controls) (Exon et al. 1984). When mice were fed 383 or 230 mg/kg/day for 90 days or 6 months, respectively, no effects were noted on serum AST or ALT activities (Borzelleca et al. 1985a, 1985c; Kobayashi et al. 1972). One of 10 mice exposed to 230 mg/kg/day 2,4-DCP for 6 months had hepatocellular hyperplasia. No liver effects were observed at 100 mg/kg/day (Kobayashi et al. 1972). No histopathological changes were observed in the livers of Fischer-344 rats fed 2,4-DCP in the diet at doses up to 2,000 mg/kg/day for 13 weeks or 400 mg/kg/day for 103 weeks (NTP 1989). Liver weights or liver enzymes released to the serum were not measured in the NTP (1989) study. Mice fed 325 mg/kg/day of 2,4-DCP for 13 weeks had dose-related increases in hepatocellular necrosis (not further described) and multinucleated hepatocytes (NTP 1989). Diffuse syncytial alterations occurred in male mice given 800 mg/kg/day 2,4-DCP in the diet for 103 weeks (NTP 1989). The number of cells affected was small, and the affected cells were scattered within the histologic sections.

A single intraperitoneal injection of 2,4-DCP (120 mg/kg) to male Kunming mice resulted in significant increases in serum ALT and AST as well as histologic changes including inflammatory cell infiltration, central venous congestion, and abnormal morphology (not further detailed) in the livers at sacrifice 1, 3, or 5 days after dosing (Fu et al. 2016). These authors observed endoplasmic reticulum (ER) stress (measured as increased expression of Bip and CHOP messenger ribonucleic acid [mRNA] and proteins)

in the livers of exposed mice on days 1 and 3 postdosing. Coupled with the observation that pretreatment with sodium tauroursodeoxycholate (TUDCA, an inhibitor of ER stress) reduced the effects of 2,4-DCP on serum ALT and AST (as well as Bip and CHOP mRNA levels), these data suggest that ER stress induction plays a role in the hepatic effects of 2,4-DCP. *In vitro* assays confirmed the effects of 2,4-DCP on Bip and CHOP mRNA and protein expression in human hepatocytes (HL7702 cells). Because ER stress can trigger apoptosis, the authors performed additional *in vitro* assays to assess apoptosis. In cultured hepatocytes, 2,4-DCP exposure (0, 0.5, 0.75, or 1.0 mM) resulted in dose-dependent reductions in mitochondrial membrane potential (MMP) and increases in the percentages of apoptotic cells, providing further evidence for the role of ER stress and apoptosis in 2,4-DCP induced hepatic effects.

2,4,5-TCP. A dose of 400 mg/kg/day 2,4,5-TCP decreased microsomal cytochrome c-reductase activity and cytochrome P-450 activity in rats exposed for 14 days; ethyl-*p*-nitrophenylphosphonothionate detoxification was not affected (Carlson 1978). A similar experiment at 200 mg/kg/day 2,4,5-TCP showed no change in glucuronyltransferase activity in exposed rats (Carlson 1978). In another rat study, Kitchin and Brown (1988) examined the effects of a single gavage dose of 2,4,5-TCP on ornithine decarboxylase activity in the liver and serum ALT activity. At a 2,4,5-TCP dose of 164 mg/kg, no effects were observed on these parameters. Histologic changes in the liver were not observed when rats were treated by gavage with 2,4,5-TCP in corn oil at doses up to 1,000 mg/kg/day for 18 times in 24 days (McCollister et al. 1961). Slight pathologic changes, which were not further described, were noted in the livers of rabbits treated by gavage with 2,4,5-TCP in 5% gum acacia solution 20 times in 28 days (McCollister et al. 1961). Over a 98-day period, a dose of 300 mg/kg/day given to rats in the diet resulted in mild centrilobular degeneration and focal necrosis, with no effects observed at 100 mg/kg/day (McCollister et al. 1961).

2,4,6-TCP. The treatment of rats with 2,4,6-TCP by gavage at doses up to 400 mg/kg/day for 14 days had no effect on ethyl-*p*-nitrophenylphosphonothionate detoxification or microsomal enzyme activities (cytochrome c-reductase, cytochrome P-450, or glucuronyltransferase) (Carlson 1978). Kitchin and Brown (1988) observed a significant increase in liver ornithine decarboxylase activity, but no significant change in serum ALT in rats given a single oral dose of 2,4,6-TCP (500 mg/kg).

Increased liver weight and midzonal vacuolation of hepatocytes were evident in rats exposed orally for 7 weeks to 2,300 mg/kg/day 2,4,6-TCP (NCI 1979). Concentration-related increases in absolute liver weight occurred in weanling Sprague-Dawley rats exposed to 4.6 or 46 mg/kg/day 2,4,6-TCP from conception through weaning and in drinking water for an additional 12 weeks (Exon and Koller 1985).

The investigators did not examine functional or anatomical hepatic parameters. Increased relative liver weights and increased serum albumin and total protein were found in groups of male rats (Sprague-Dawley) exposed to 240 and 720 mg/kg/day and female rats exposed to 720 mg/kg/day of 2,4,6-TCP for 90 days (Bercz et al. 1990). Males administered 720 mg/kg/day also had increased serum ALT. The investigators attributed clinical chemistry results to either an altered hydration status or dysfunctional hepatic activity; no treatment-related histopathological evidence of tissue damage was noted in either sex (Bercz et al. 1990). The investigators considered 240 mg/kg/day as a LOAEL for hepatic effects and the next lower dose, 80 mg/kg/day, as a NOAEL for intermediate-duration exposure. In contrast to effects seen in Sprague-Dawley rats, increased liver weight and histopathologic lesions were not evident in Long-Evans or F344 rats exposed to 2,4,6-TCP over intermediate or chronic periods at doses up to 1,000 and 500 mg/kg/day, respectively (Blackburn et al. 1986; NCI 1979).

Microscopic examination revealed hepatic hyperplasia and other signs of hepatocellular damage (e.g., liver cell abnormalities, focal areas of cellular alteration) in mice exposed chronically to 2,4,6-TCP in the diet at doses as low as 650 mg/kg/day (NCI 1979).

The differing hepatic effects of 2,4,6-TCP in available studies may, in part, be a result of the different methodologies used for exposure, variations in experimental design (including different species and strains), and/or possible differences in gastrointestinal absorption because of the nature of the vehicle. In the intermediate oral studies by Bercz et al. (1990) and Blackburn et al. (1986), 2,4,6-TCP was administered in corn oil by gavage. Interpretation of the Blackburn et al. (1986) data is further complicated by the investigators' failure to report sample sizes used in the statistical analysis. The NCI (1979) studies administered 2,4,6-TCP in the diet, while 2,4,6-TCP was administered in drinking water in the Exon and Koller (1985) study, rendering direct comparisons uncertain.

2,3,4,6-TeCP. In the study by Kitchin and Brown (1988), 2,3,4,6-TeCP administration as a single dose (193 mg/kg) to rats induced an increase in ornithine decarboxylase activity in the liver without a significant change in serum ALT. In a comprehensive examination of the hepatic effects of 2,3,4,6-TeCP, Sprague-Dawley rats were administered up to 200 mg/kg/day 2,3,4,6-TeCP by gavage (5 days/week) for 13 weeks, with interim sacrifices after 5 days (acute) and 2 and 4 weeks (intermediate) (Dodd et al. 2012). After 5 days of exposure, an increase in liver weight was seen at 100 and 200 mg/kg/day, and an increased incidence of centrilobular hypertrophy was noted at 200 mg/kg/day group. After 2 weeks of exposure, significant increases (≥14% compared to controls) in absolute and relative liver weight were observed at doses ≥25 mg/kg/day, and statistically significant increased incidences of histopathology

changes in the liver (centrilobular hypertrophy and necrosis) were observed at ≥50 mg/kg/day. After 4 weeks, increased incidences of hepatic centrilobular vacuolation and hypertrophy were seen at doses ≥25 mg/kg/day, and significant (≥21%) increases in absolute and relative liver weights occurred at ≥50 mg/kg/day. At the end of the study after 13 weeks of exposure, significant (≥41%) increases in absolute and relative liver weight were reported at all but the lowest dose. Centrilobular vacuolation was seen in all groups including controls (4/12), but the incidence and severity increased with dose such that all animals were affected at doses ≥25 mg/kg/day. Hypertrophy was not seen in controls, but the incidence increased with dose from 4/10 at 10 mg/kg/day to all animals (9/9 or 10/10) at doses of at least 50 mg/kg/day. Necrosis was observed at doses of ≥50 mg/kg/day, from 3/9 at 50 mg/kg/day to 10/10 at 200 mg/kg/day. Incidences of other histopathology lesions were not reported in tables. All high-dose (200 mg/kg/day) rats exhibited bile duct hyperplasia, as did 20% of rats in the 100 and 25 mg/kg/day groups. Finally, centrilobular and/or periportal fibrosis was observed at 10% incidence in groups exposed to 25 and 100 mg/kg/day and at 40–60% incidence in the 200 mg/kg/day group (Dodd et al. 2012).

In a study sponsored by the EPA (1986), increased liver weights (>20 and >16% for absolute and relative weights, respectively) in males and centrilobular hypertrophy were observed in rats administered 100 or 200 mg/kg/day 2,3,4,6-TeCP by gavage for 90 days. No effects were observed at 25 mg/kg/day. In an intermediate-duration study (55 days) with limited reporting, gavage administration of 100 mg/kg/day to Wistar rats resulted in moderate to severe hepatic damage (consisting of bile duct proliferation, focal necrosis, and polymorphonuclear leukocyte infiltration or large necroses with dilated and thrombosed veins; incidences not reported). At 50 mg/kg/day, 1 out of 10 rats showed severe damage, and at 10 mg/kg/day, no liver effects were seen (Hattula et al. 1981).

2.10 RENAL

No studies were located regarding renal effects in humans after exposure to any of the chlorophenols discussed in this profile. Animal studies suggest that acute- and intermediate-duration oral exposures to mono- and dichlorophenols have little effect on kidney endpoints. Intermediate-duration studies indicated effects on the kidneys (mild degenerative changes on the tubular epithelium or increased kidney weights) in rats exposed to 2,4,5-TCP (McCollister et al. 1961) or 2,3,4,6-TeCP (EPA 1986).

2-CP. In mice, daily administration of 35 or 69 mg/kg/day 2-CP for 14 days had no adverse effects on measures of renal function, including BUN, total protein, albumin/globulin ratio, or electrolyte balance (Borzelleca et al. 1985a). No significant compound-related adverse effects were noted in the kidney at

necropsy; however, a dose of 175 mg/kg/day was lethal to all exposed mice. When Sprague-Dawley rats were exposed to 2-CP by gavage for 10 days (at doses up to 257 mg/kg/day) or 90 days (at doses up to 150 mg/kg/day), no effects on BUN, serum creatinine, kidney weight, or kidney histology were noted (Daniel et al. 1993).

No exposure-related effects on serum chemistry, urinalysis parameters, kidney weight, or microscopic kidney findings were observed in Sprague-Dawley rats given 2-CP by gavage at doses up to 1,000 mg/kg/day for 28 days (Hasegawa et al. 2005). By contrast, neonatal rats given 300 mg/kg/day 2-CP for 18 days exhibited increased incidences of basophilic renal tubules, in the absence of changes in serum chemistry, urinalysis, or kidney weight (discussed further in Section 2.17).

4-CP. An increase in relative kidney weight, in the absence of effects on body weight, was observed in female ICR mice 7 days after a single gavage dose of 1,575 mg/kg 4-CP; 4/10 mice receiving this dose died (Shi et al. 2013). In a 28-day study, Sprague-Dawley rats exposed to 4-CP by gavage at doses up to 500 mg/kg/day exhibited no treatment-related effects on serum chemistry, urinalysis parameters, kidney weight, or kidney histology (Hasegawa et al. 2005).

2,4-DCP. No significant changes in serum BUN or creatinine were observed in BALB/c mice given 1,000 ppm 2,4-DCP (270 mg/kg/day) in drinking water for 14 days (Aydin et al. 2009). Except for renal tubular necrosis in mice that died following treatment with 2,4-DCP in the diet for 3 weeks at 5,200 mg/kg/day (NTP 1989), kidney effects have not been observed in intermediate-duration studies of animals treated with 2,4-DCP. Based on histological examinations, the NOAELs for kidney effects after dietary exposure to 2,4-DCP are 2,000 and 440 mg/kg/day for rats exposed for 13 and 103 weeks, respectively (NTP 1989); and 230, 2,600, and 1,300 for mice exposed for 90 days, 13 weeks, and 103 weeks, respectively (Kobayashi et al. 1972; NTP 1989). Treatment of mice for 90 days with 2,4-DCP in drinking water at doses up to 491 mg/kg/day (in females) or 383 mg/kg/day (in males) had no effect on kidney weights or clinical chemistry values including urine protein, phosphorus, calcium, sodium, chloride, potassium, or creatinine levels; histopathological examinations were not completed (Borzelleca et al. 1985a, 1985c).

2,4,5-TCP. Treatment of rats with 2,4,5-TCP (1,000 mg/kg/day by gavage) for 18 days resulted in a significant increase in kidney weight, with no histopathologic changes or changes in BUN (McCollister et al. 1961). Slight pathologic changes (not further described) were observed in rabbits given 20 gavage doses of 100 or 500 mg/kg/day, with no effects noted at 10 mg/kg/day (McCollister et al. 1961). In a

98-day study, 2,4,5-TCP administered in the diet at 300 mg/kg/day resulted in mild degenerative changes in the renal epithelium of the convoluted tubules and in proliferation of the interstitial tissue (McCollister et al. 1961). No kidney effects were observed at 100 mg/kg/day.

2,4,6-TCP. Administration of 720 mg/kg/day 2,4,6-TCP in corn oil by gavage for 90 days resulted in increased absolute and relative kidney weights in male, but not female, Sprague-Dawley rats, as well as decreased urinary pH in both sexes. No other effects on clinical parameters of renal function were observed (Bercz et al. 1990). Renal weight was not increased in Long-Evans rats administered 2,4,6-TCP in corn oil by gavage at doses as high as 1,000 mg/kg/day for 11 weeks, 5 days/week (Blackburn et al. 1986). Strain differences and daily treatment as opposed to treatment 5 times/week may account for the differences in renal effects in the Bercz et al. (1990) and Blackburn et al. (1986) studies. No treatment-related lesions were evident upon histopathologic examination of the kidney in rats and mice exposed to dietary 2,4,6-TCP for 2 years at doses as high as 500 and 1,356 mg/kg/day, respectively (NCI 1979).

2,3,4,6-TeCP. Neither a single dose nor 55-day repeated exposure to 2,3,4,6-TeCP at doses up to 632 mg/kg or 100 mg/kg/day, respectively, induced adverse effects on the histological appearance of the kidneys of rats (Hattula et al. 1981). Increased kidney weights without any histopathologic changes were observed in rats treated by gavage with 2,3,4,6-TeCP at 100 mg/kg/day, but not at 25 mg/kg/day, for 90 days (EPA 1986).

2.11 DERMAL

Chloracne and evidence of acquired porphyria, hyperpigmentation, and hirsutism have been observed in workers employed in the manufacture of 2,4-DCP- and 2,4,5-TCP-based herbicides (Bleiberg et al. 1964; Bond et al. 1989). The chloracne incidence was greatest in young employees exposed in trichlorophenol production and in chlorophenol production and finishing procedures (Bond et al. 1989). In this study, workers exposed to the highest concentration of the contaminant TCDD were at the greatest risk of developing chloracne.

The results of available animal studies indicate that chlorophenols are damaging to epithelial tissue.

2-CP and 4-CP. Severe effects have been reported at exposure levels of 242–2,000 mg/kg of 2-CP or 4-CP applied directly to rabbit skin (Rhone-Poulenc 1978, 1981). Corrosion (not further described) was typically accompanied by other signs of severe skin injury, including erythema, edema, and discoloration.

A single dermal application of a lower dose (100 mg/kg) of 4-CP to one ear of a mouse did not increase ear weight relative to the untreated ear (Dohi et al. 1989).

2,4-DCP. Dermal lesions were caused by a single direct application of as little as 200 mg/kg 2,4-DCP to bare abdominal skin of New Zealand White rabbits (Carreon et al. 1980a, 1980b; Hencke and Lockwood 1978; Monsanto 1976). The dose-related dermal damage observed was described as mild-to-moderate erythema and mild-to-marked edema, followed by necrosis and scabbing.

2,3,4,5-TeCP. Dermal application of 20 mL/kg (32 g/kg) 2,3,4,5-TeCP on the shaved skin of female rats resulted in dermatosis associated with scar formation. Rats treated with a sodium hydroxide-extracted fraction of 2,3,4,5-TeCP had no dermatological lesion, indicating that the adverse effects were attributable to the chlorophenol rather than contaminants, such as dioxins (Shen et al. 1983).

Mechanisms. Corrosive skin damage resulting from high-concentration chlorophenol exposure has been attributed to protein denaturation by protein-solute complexes (Roberts et al. 1977). In this study, various concentrations of 2-CP and 4-CP were applied to samples of human abdominal skin maintained in a diffusion chamber. The estimated threshold concentrations for damage (the aqueous concentration at which the transmembrane permeability coefficient began to increase) were 0.8 and 0.75%, respectively, for these two isomers. The investigators proposed that the extent of damage was related to the concentration of the solute partitioned into the stratum corneum, the diffusivity of the solute, and the pK of the applied compound.

2.12 OCULAR

Lumber mill workers exposed to a mixture of tetrachlorophenols (specific isomers not stated) and pentachlorophenol reported eye irritation more frequently than unexposed workers (Kleinman et al. 1986). The eye irritation may have resulted from contact of the eye with the airborne chemicals or contact with contaminated surfaces (e.g., hands, clothing). Animal studies indicate that direct application of chlorophenols to the eyes can induce severe damage, but oral exposure does not affect the eyes.

2-CP. Severe discomfort and corrosion was reported to occur 1 minute after the application of 33 mg/kg undiluted 2-CP to rabbit eyes (Monsanto 1975).

4-CP. When 0.6 mg/kg 4-CP (as a 1% solution) was applied to the corneas of rabbits, slight hyperemia was noted (Harrison and Madonia 1971). At 1.2 mg/kg, rabbits had more severe hyperemia, with edematous swelling, corneal cloudiness, and exudation. The maximum response occurred 5 hours after application, and inflammation was no longer apparent at 96 hours (Harrison and Madonia 1971).

2,4-DCP. Severe corneal damage occurred in the eyes of rabbits after a single direct application of 0.1 mL 2,4-DCP (Hencke and Lockwood 1978). Careful washing of the eye 30 seconds after application did not prevent this damage.

In rats and mice treated with 2,4-DCP in the diet for intermediate or chronic durations, histopathologic examination of the eyes did not reveal any adverse effect (NTP 1989).

2,4,6-TCP. Ophthalmoscopic examinations did not reveal any treatment-related effects in rats treated with 2,4,6-TCP by gavage at doses up to 720 mg/kg/day for 90 days (Bercz et al. 1990).

2,3,4,6-TeCP. Histopathologic examination of the eyes did not reveal any adverse effect in rats exposed to 2,3,4,6-TeCP by gavage at doses up to 200 mg/kg/day for 90 days (EPA 1986).

2.13 ENDOCRINE

Available human studies of endocrine effects of chlorophenols have used urinary levels of chlorophenols to assess exposure. However, chlorophenols in urine can occur as a result of metabolism of other compounds such as chlorinated benzenes (see Section 3.3.1 for further information); thus, the relevance of these studies to hazard identification is uncertain. Animals exposed orally to chlorophenols have not shown effects on endocrine organ weights or microscopic findings in these organs.

In a nested case-control study of pregnant women participating in the Lifecodes longitudinal birth cohort, urine and blood samples were collected 4 times during pregnancy for measurement of dichlorophenols and thyroid hormone levels, respectively (Aker et al. 2018). Repeated measures analyses showed no associations between 2,4-DCP or 2,5-DCP in urine and serum levels of thyroid stimulating hormone (TSH), free thyroxine (FT4), thyroxine (T4), or triiodothyronine (T3) (Aker et al. 2018). When results were stratified by gestational age, urinary 2,4-DCP levels showed associations with decreased T3 and marginally increased TSH at 21–30 weeks of gestation, as well as marginally decreased TSH at <21 weeks of gestation (Aker et al. 2018). Marginal associations between 2,5-DCP and serum TSH were

observed, but the direction of change was not consistent across gestational periods. In a similar study, concentrations of 2,4-DCP in urine measured twice during pregnancy were inversely associated with free T4 in maternal serum, but not with total T4 in maternal serum or with TSH levels in serum of mothers or their neonates in a study of 338 mothers participating in a longitudinal birth cohort study (Center for the Health Assessment of Mothers and Children of Salinas or CHAMACOS) (Berger et al. 2018). Urinary 2,5-DCP levels were not associated with these endpoints (Berger et al. 2018).

Urinary concentrations of 2,5-, but not 2,4-DCP were associated with higher prevalence of hypothyroidism among 618 adolescents (ages 12–19 years) participating in NHANES surveys during 2007–2008 and 2011–2012 (Wei et al. 2016). The authors noted that urinary 2,5-DCP was considered to be a reliable biomarker for *p*-dichlorobenzene exposure; thus, the relationship to hazard identification for 2,5-DCP is uncertain. No association was observed between serum concentrations of 2,4,5,6-TeCP and transthyretin-bound thyroxin in a cross-sectional study of 120 adult (ages 18–39 years) Inuit women (Audet-Delage et al. 2013). Rooney et al. (2018) did not observe an association between urinary 2,4- or 2,5-DCP levels and thyroid problems (not further specified) in a cross-sectional study of 3,617 adult NHANES (2007–2010) participants.

2-CP. In Sprague-Dawley rats given 2-CP by gavage, no changes in adrenal weight or histology of adrenal glands, pancreas, pituitary, or thyroid/parathyroid glands were observed at doses up to 257 mg/kg/day for 10 days or up to 150 mg/kg/day for 90 days (Daniel et al. 1993). Likewise, 28-day exposure of Sprague-Dawley rats to doses up to 1,000 mg/kg/day via gavage did not result in changes in the weights or histology of adrenal, pituitary, or thyroid glands (Hasegawa et al. 2005).

4-CP. When Sprague-Dawley rats received doses up to 500 mg/kg/day 4-CP by gavage for 28 days, there were no changes in endocrine organ weights (adrenal, pituitary, and thyroid glands) or microscopic findings in these organs (Hasegawa et al. 2005).

2,4-DCP. Histopathologic examinations did not reveal any changes in the endocrine glands (adrenals, pituitary, thyroid, pancreas) of rats or mice treated with 2,4-DCP in the diets at doses up to 2,000 (rats) or 2,600 (mice) mg/kg/day for 13 weeks, or at doses up to 440 (rats) or 1,300 (mice) mg/kg/day for 103 weeks (NTP 1989).

2,4,5-TCP. No histopathologic changes were observed in the adrenals of rats exposed to 2,4,5-TCP in the diet at 1,000 mg/kg/day for 98 days (McCollister et al. 1961).

2,4,6-TCP. Female rats treated by gavage with 720 mg/kg/day of 2,4,6-TCP for 90 days had slightly, but statistically significant, increases in adrenal weights compared to untreated controls, without concomitant histopathological changes (Bercz et al. 1990). Adrenal gland weights were not increased in male rats treated by gavage with 2,4,6-TCP at 1,000 mg/kg/day for 11 weeks (Blackburn et al. 1986). Histopathologic changes were not observed in the adrenal glands, thyroid, pancreas, or parathyroid glands in rats or mice treated with 2,4,6-TCP in the diet at doses of 500 (rats) or 1,356 (mice) mg/kg/day for 105 weeks (NCI 1979).

2,3,4,6-TeCP. Treatment of rats by gavage with 2,3,4,6-TeCP for 90 days at doses up to 200 mg/kg/day had no effect on the histologic appearance of the adrenal glands, pituitary, pancreas, or thymus (EPA 1986).

Mechanisms. The potential for 2,4-DCP to affect thyroid hormone functions was evaluated in an *in vitro* study using isolated T3, recombinant protein disulfide isomerase (PDI; an intracellular thyroid hormone binding protein that assists in protein folding), and recombinant nuclear thyroid hormone receptor (Okada et al. 2005). 2,4-DCP produced dose-dependent inhibition of PDI activity, PDI-T3 binding, and T3-nuclear thyroid hormone receptor binding. Results indicate that 2,4-DCP may alter thyroid function through changes in intracellular processing of T3 (Kim et al. 2005). None of the three chlorophenols (2-CP, 2,4-DCP, and 2,4,6-TCP) tested for agonistic and antagonistic activity in a thyroid receptor β transcriptional assay exhibited any activity. Yang et al. (2021) conducted *in vitro* experiments measuring the potential binding of three chlorophenols (2-CP, 2,3-DCP, and 2,4,6-TCP) to human transthyretin (hTTR), a protein that plays an important role in thyroid hormone distribution. The aim of the study was to compare the binding affinity of chlorinated phenols to that of chlorinated thiophenols. Using a cell-free competitive fluorescence displacement assay, the study authors detected weak binding in experiments with 2-CP and 2,3-DCP, but no binding with 2,4,6-TCP (Yang et al. 2021).

2.14 IMMUNOLOGICAL

Available human studies of immunological effects of chlorophenols have used urinary 2,4- and 2,5-DCP levels to measure exposure. However, as noted earlier, urinary chlorophenols may result from metabolism of other compounds, and in particular 2,5-DCP in urine is considered to be a reliable biomarker for exposure to *p*-dichlorobenzene (Yoshida et al. 2002). Of the three chlorophenols (2-CP, 2,4-DCP, and 2,4,6-TCP) tested for sensitive measures of immunotoxicity in animals exposed orally, only

2,4-DCP showed evidence of adverse effects. In rats, 2,4-DCP exposure (≥4.6 mg/kg/day in drinking water) resulted in decreased delayed-type hypersensitivity, and higher doses induced increased serum antibodies to keyhole limpet hemocyanin (Exon and Koller 1985; Exon et al. 1984).

In a cross-sectional study, Vindenes et al. (2021) observed no association between urinary 2,4- or 2,5-DCP and self-reported prevalence of eczema, rhinitis, or asthma in 496 adults in Norway. The investigators also collected blood samples from participants and analyzed the samples for specific IgE to five allergens including cat, timothy grass, Cladosporium, birch, or house dust. A significant positive association was noted between urinary 2,4-DCP and specific IgE to at least one of the five allergens (change in specific IgE of 0.15 based on multiple linear regression analysis); no association was seen with 2,5-DCP.

Two studies (Aung et al. 2019; Watkins et al. 2015) evaluated whether urinary levels of 2,4- or 2,5-DCP in pregnant women were associated with serum markers of inflammation. Watkins et al. (2015) included a total of 54 subjects (participants in the Puerto Rico Testsite for Exploring Contamination Threats or PROTECT project) who provided urinary samples three times and blood samples twice during pregnancy. Linear mixed models were used to account for intraindividual correlation across sampling times. No association was observed between serum measures of inflammation (interleukins [IL-1β, IL-6, IL-10], tumor necrosis factor [TNF-α], or C-reactive protein [CRP]) and specific gravity-adjusted levels of 2,4- or 2,5-DCP in urine (Watkins et al. 2015). Aung et al. (2019) evaluated the same inflammatory markers in a nested preterm birth case-control study (participants in the LIFECODES prospective birth cohort) of 130 cases and 352 controls, each of whom provided urine samples four times and blood samples five times during pregnancy. Linear mixed models analysis did not indicate any association between serum IL-1β, IL-6, IL-10, TNF-α, or CRP and urinary concentrations of 2,4- or 2,5-DCP (Aung et al. 2019). Serum CRP levels were positively associated (10% increase in serum CRP with interquartile increase in urinary concentration) with urinary 2,5-DCP, but not urinary 2,4-DCP, in this study (Aung et al. 2019).

In a case report of contact dermatitis associated with clothing, Pesqué et al. (2021) obtained ethanol and acetone extracts of the fabric the woman wore and used gas chromatography-mass spectrometry (GC-MS) analysis and patch testing to identify 2,4-DCP as the likely cause. Negative patch tests with other compounds isolated from the fabric supported the characterization of 2,4-DCP as the causative agent.

2-CP. Mice fed 69 mg/kg/day 2-CP for 14 days showed no changes in humoral or cell-mediated immunological assays (Borzelleca et al. 1985a). Statistically significant decreases in spleen weight were

noted at 69 mg/kg/day, but no gross abnormalities in spleen morphology were observed (Borzelleca et al. 1985a). At the next higher dose (175 mg/kg/day), all mice died prematurely. A 10-day exposure to 2-CP via gavage (at doses up to 257 mg/kg/day) in Sprague-Dawley rats did not alter spleen or thymus weights or histology (Daniel et al. 1993).

Rats fed 73 mg/kg/day 2-CP from conception through weaning and for an additional 10 weeks showed no changes in humoral or cell-mediated immunological assays including tests for antibody production, delayed-type hypersensitivity, or phagocytic activity of peritoneal exudate cells (Exon and Koller 1983b, 1985). Furthermore, neither thymus nor spleen weights were affected by exposure (Exon and Koller 1983b, 1985). Similarly, no effects on thymus or spleen weights or histopathology were noted in Sprague-Dawley rats given gavage doses up to 1,000 mg/kg/day 2-CP for 28 days (Hasegawa et al. 2005) or up to 150 mg/kg/day 2-CP for 90 days (Daniel et al. 1993).

4-CP. Spleen and thymus weights were not affected by exposure to 4-CP doses up to 300 mg/kg/day for 28 days in Sprague-Dawley rats, and there were no histopathology findings in these organs (Hasegawa et al. 2005).

2,4-DCP. Sensitivity tests have demonstrated immune system effects in animals exposed to low doses of 2,4-DCP administered for 15 weeks. Decreased delayed-type hypersensitivity occurred in rats exposed to 4.6 mg/kg/day of 2,4-DCP in drinking water, and increased serum antibodies to keyhole limpet hemocyanin were found in the blood of rats during similar exposures to 46 mg/kg/day (Exon and Koller 1985; Exon et al. 1984). Macrophage function, measured by the *in vitro* phagocytosis of sheep red blood cells, showed no effect from 2,4-DCP treatment. No immune system effects occurred with exposure to 0.46 mg/kg/day (Exon and Koller 1985; Exon et al. 1984).

In contrast, organ weight and histopathology examinations have not generally shown evidence of alterations in the immune system after intermediate-duration exposure. Spleen weights were nearly doubled (compared to controls) but thymus weights were not significantly affected in rats that received 46 mg 2,4-DCP kg/day from conception through weaning (via maternal dosing) and for an additional 15 weeks in drinking water (Exon and Koller 1983b, 1985). Histopathological examination of lymph nodes, spleen, and thymus did not reveal any effects in rats or mice treated with 2,4-DCP in the diet at doses up to 2,000 mg/kg/day (rats) and 2,600 mg/kg/day (mice) for 13 weeks (NTP 1989). Bone marrow atrophy was observed in rats treated at 500 mg/kg/day, but not 250 mg/kg/day, for 13 weeks (NTP 1989). Because both erythroid and myeloid elements were affected, this study is also discussed in Section 2.7

(Hematological). No changes in spleen weight were observed in mice treated with 2,4-DCP in the diet at 230 mg/kg/day for 6 months (Kobayashi et al. 1972), and no changes in spleen or thymus weight were noted in mice treated with 2,4-DCP in the drinking water at doses up to 491 mg/kg/day (in females) or 383 mg/kg/day (in males) for 90 days (Borzelleca et al. 1985a, 1985c).

Chronic (103 week) exposure to 2,4-DCP in the diet did not induce histopathological changes in the lymph nodes, spleen, or thymus in rats or mice treated at doses up to 440 (rats) and 1,300 mg/kg/day (mice) (NTP 1989).

2,4,5-TCP. In rats treated with 2,4,5-TCP in the diet at doses of 1,000 mg/kg/day for 98 days, spleen weight and histological appearance were not altered by treatment (McCollister et al. 1961).

The murine local lymph node assay, which is predictive of skin sensitization potential, was completed in mice treated with 2,4,5-TCP (Kimber and Weisberger 1991). A single dermal exposure of 50 mL of 2,4,5-TCP was applied on one shaved flank; 5 days later, the mice were given three daily doses (140–560 mg/kg/day) applied to the ear. A positive response was observed at all doses, suggesting that 2,4,5-TCP can be a skin sensitizer. This study is limited since only three mice were used in each group and a statistical analysis of the data was not completed.

2,4,6-TCP. No changes in spleen weight or histological appearance were observed in rats treated by gavage with 720 mg/kg/day 2,4,6-TCP for 90 days (Bercz et al. 1990). Spleen weights were significantly increased in rats exposed to 2,4,6-TCP in the drinking water both pre- and postnatally (~15 weeks postweaning) at doses of 46 mg/kg/day, while no significant effects on immune function (antibody levels, delayed-type hypersensitivity, macrophage numbers) were observed (Exon and Koller 1985). Treatment of rats and mice with 2,4,6-TCP in the diet for 2 years at doses up to 500 mg/kg/day for rats and 1,356 mg/kg/day for mice did not reveal any significant gross or histopathological changes in the spleen, lymph nodes, or thymus (NCI 1979).

2,3,4,6-TeCP. Administration of a single gavage dose 632 mg/kg of 2,3,4,6-TeCP in Wistar rats resulted in “slight stasis” in the spleens of rats (Hattula et al. 1981); the toxicological significance of this finding is unknown. No histological changes were observed in the spleen, lymph nodes, or thymus of rats treated with 2,3,4,6-TeCP by gavage at doses up to 200 mg/kg/day for 90 days (EPA 1986).

Mechanisms. There are few studies examining potential mechanisms of chlorophenol-induced immune system effects. Xie et al. (2019) compared the cytotoxicity of three trihalogenated phenols (2,4,6-TCP, tribromophenol, and triiodophenol) in mouse macrophage RAW264.7 cells. All three compounds induced cell proliferation at low concentrations (200 μM) and cytotoxicity at higher concentrations (≥300 μM). Morphological assessment and flow cytometry showed that 2,4,6-TCP exposure induced M1 polarization (resulting in a pro-inflammatory macrophage phenotype) at 200 μM. The pro-inflammatory response was supported by the observation of dose-related increases in the mRNA transcription of the M1 marker, inducible nitric oxide synthase (iNOS), and a slight increase in mRNA level of tumor necrosis factor-alpha (TNF-α). Measurement of protein levels in the cells using enzyme-linked immunosorbent assay (ELISA) showed dose-related decreases in the production of TNF-α and interleukin-6 (IL-6). The study authors did not discuss potential reasons for the discrepancy between observed effects on TNF-α mRNA and protein levels. Taken together, however, the results of the study suggest that 2,4,6-TCP could induce a pro-inflammatory response in mouse macrophages.

2.15 NEUROLOGICAL

Data pertaining to neurological effects of chlorophenols in humans are subject to the same limitations noted for other endpoints; these include potential confounding by coexposures to other compounds, poor exposure characterization, and/or use of nonspecific, unreliable biomarkers (e.g., urinary chlorophenol levels) to assess exposure. Lumber mill workers exposed to a mixture of tetrachlorophenols (specific isomers not stated) and pentachlorophenol reported headaches more frequently than unexposed workers (Kleinman et al. 1986). Monitoring of air and urinary concentrations of tetrachlorophenols suggested that exposure was principally through the skin, with some possibility of oral ingestion. An industrial waste worker who accidentally splashed pure 2,4-DCP on portions of his right arm and leg, experienced a seizure within 20 minutes of the exposure, and died shortly thereafter (Kintz et al. 1992).

Several cross-sectional studies examined relationships between di- or trichlorophenol levels in urine and prevalence of self-reported neurological effects on olfaction, vision, hearing, balance, or attention deficit hyperactivity disorder (ADHD) among NHANES participants. In a study of 10,122 adults >50 years of age participating in the 2003–2004 NHANES survey, Shiue (2013) evaluated self-reported vision, hearing, and balance problems. After adjustment for covariates, an increased odds of self-reported balance problems (dizziness, falling) was observed with higher urinary 2,4,5-TCP, and an increased odds of reporting ringing, roaring, or buzzing in the ears was associated with higher urinary 2,4-DCP. No association with vision, hearing, or balance problems was observed for 2,5-DCP or 2,4,6-TCP (Shiue et

al. 2013). Among respondents in the 2013–2014 NHANES survey who participated in an odor detection test (the NHANES Pocket Smell Test), an increased odds of hyposmia (scoring 4–5 on the test) was associated with higher urinary levels of 2,4-DCP when compared with levels among those scoring as normal (scores of 6–8) (Bello and Dumancas 2017; Noel et al. 2017). Urinary concentrations of 2,4-DCP were not reported, nor were results for other chlorophenols. Xu et al. (2011) evaluated the association between trichlorophenol exposure and attention deficit disorders in 2,546 children aged 6–15 years participating in the 1999–2004 NHANES survey. The results showed that children with low (<3.58 μg/g) and high (≥3.58 μg/g) levels of 2,4,6-TCP in urine samples had higher risks of parent-reported attention deficit disorder than children with urinary 2,4,6-TCP levels below the levels of instrumentation detection. No association was seen with urinary levels of 2,4,5-TCP (Xu et al. 2011).

As described below, high doses of chlorophenols have resulted in clinical signs of neurotoxicity in animals. Lethargy, tremors, convulsions, and/or central nervous system depression have been reported in animals exposed orally or dermally to 2- and 4-CP and 2,4-DCP (Borzelleca et al. 1985a; Carreon et al. 1980a, 1980b; Hasegawa et al. 2005; Monsanto 1976; NTP 1989; Phornchirasilp et al. 1989b; Rhone-Poulenc 1991; Spencer and Williams 1950) or to tetrachlorophenols via single dermal application (Shen et al. 1983). The lowest dose associated with neurotoxicity after exposure for any duration was 35 mg/kg/day 2-CP in an acute-duration study of mice (Borzelleca et al. 1985a). No studies evaluating more sensitive measures of neurological function in animals exposed to any of the subject chlorophenols were identified in the available literature.

2-CP. In an LD_{50} study, single oral doses (unspecified) of 2-CP caused motor weakness, tremors, convulsions, and central nervous system depression in rats and mice (Borzelleca et al. 1985a, 1985b). The actual doses used in the study (Borzelleca et al. 1985b) were not stated. Single oral doses of 2-CP >300 mg/kg resulted in distress and twitching in rabbits (Spencer and Williams 1950). Clinical signs were not observed and neither brain weight nor sciatic nerve histology was affected by exposure in Sprague-Dawley rats treated by gavage to doses up to 257 mg/kg/day 2-CP for 10 days (Daniel et al. 1993).

When rats were exposed by nose-only inhalation for 4 hours to 908 ppm 2-CP, signs of toxicity included restlessness, a hunched posture, and ruffled fur (Rhone-Poulenc 1991). These effects were not observed at 104 ppm.

Newborn rats (12/sex/group) administered 2-CP in olive oil by gavage at or 300 mg/kg/day on PNDs 4–21 (18 days total) exhibited significant increases in the incidence of tremors (11/12 males, 12/12 females); few signs of hypoactivity or abnormal gait were observed (Hasegawa et al. 2005). Although one female exhibited tremors at 50 mg/kg/day, no other animals exposed to lower doses (up to 100 mg/kg/day) exhibited clinical signs of neurotoxicity. The clinical signs of neurotoxicity appeared within 5 minutes of dosing and vanished approximately 4 hours post-exposure (Hasegawa et al. 2005). In a related experiment reported in the same publication, young (5–6 weeks old) Sprague-Dawley rats treated with 1,000 mg/kg/day 2-CP in olive oil for 28 days showed tremors (9/24), hypoactivity (13/24), and abnormal gait (11/24). The signs of neurotoxicity appeared approximately 3 hours after dosing; times to disappearance of symptoms were not reported (Hasegawa et al. 2005). Neither newborn nor young rats exhibited effects on brain weight or microscopic findings related to 2-CP exposure in these experiments (Hasegawa et al. 2005). Sprague-Dawley rats exposed by gavage to doses up to 150 mg/kg/day 2-CP for 90 days showed no changes in brain weight or histopathology in the brain or sciatic nerve (Daniel et al. 1993).

In male and female ICR mice, repeated oral administration of 35 and 69 mg/kg/day 2-CP for 14 days resulted in hyperactivity and decreased brain weight, respectively, but the brain tissue appeared grossly normal (Borzelleca et al. 1985a).

4-CP. Single oral doses (unspecified) of 4-CP administered to rats and mice to assess acute lethality caused motor weakness, tremors, convulsions, and central nervous system depression (Borzelleca et al. 1985a, 1985b). A single oral dose of 514 mg/kg 4-CP produced seizures immediately followed by death in male ICR mice (Phornchirasilp et al. 1989b). Spencer and Williams (1950) reported distress and twitching in rabbits after administration of single (unspecified, but reported to be >300 mg/kg) oral doses of 4-CP.

Newborn rats (12/sex/group) were administered 4-CP at doses of 0, 12, 60, or 300 mg/kg/day in olive oil by gavage on PNDs 4–21 (Hasegawa et al. 2005). Rats of both sexes treated with 300 mg/kg of 4-CP exhibited tremors (24/24), rapid breathing, and salivation; the animals were not affected at 60 mg/kg/day. Tremors occurred approximately 15–60 minutes after dosing and disappeared within 4 hours post-exposure. In a companion experiment, young (5–6 weeks old) Sprague-Dawley rats of both sexes treated with 500 mg/kg/day 4-CP by gavage for 28 days showed clinical signs of toxicity, which included tremors, rapid breathing, and salivation. The onset of symptoms occurred approximately 5–30 minutes after dosing, and the time to disappearance of symptoms was not reported (Hasegawa et al. 2005). No

change in brain weight or histology was noted in either of these experiments (Hasegawa et al. 2005). In an intermediate-duration (42–53 days) reproductive/developmental toxicity screening study in rats exposed by gavage, clinical signs of neurotoxicity, including ataxia, tremors, and clonic convulsions were observed within 30 minutes of dosing with 1,000 mg/kg/day; this dose was also lethal in about half of exposed animals (BSRC 2011). Signs of neurotoxicity were not observed at lower doses in this study (40 and 200 mg/kg/day).

2,4-DCP. Rabbits given single dermal applications of 250 mg/kg 2,4-DCP or more became lethargic (Carreon et al. 1980a, 1980b; Monsanto 1976), and two rabbits in the 2,000-mg/kg group and one in the 4,000-mg/kg group became anorexic (Carreon et al. 1980b). Lethargy was also seen in mice treated with 2,4-DCP in the diet at 5,200 mg/kg/day for 14 days; one out of five male mice died after exposure to this dose (NTP 1989).

In intermediate- and chronic-duration studies, there was little evidence for neurotoxicity after exposure to 2,4-DCP. Hunched posture was observed in rats treated with 2,4-DCP in the diet at 2,000 mg/kg/day for 13 weeks (NTP 1989) with no histopathological changes in the brain, sciatic nerve, or spinal cord. In mice treated with 2,4-DCP in the diet at doses up to 2,600 mg/kg/day for 13 weeks, no histopathological changes were observed in the brain, sciatic nerve, or spinal cord (NTP 1989). No effect on brain weight was observed in mice treated for 90 days with 2,4-DCP in the drinking water at doses up to 491 mg/kg/day (in females) or 383 mg/kg/day (in males) (Borzelleca et al. 1985a, 1985c). No clinical signs of neurological effects were reported in rats or mice fed doses up to 440 mg/kg/day for rats and 1,300 mg/kg/day for 2 years, and histopathologic examination of the brains of these animals did not reveal any effects (NTP 1989).

2,4,5-TCP. No changes in brain weight or histological appearance of the brain were observed in rats treated with 2,4,5-TCP in the diet at doses up to 1,000 mg/kg/day for 98 days (McCollister et al. 1961).

2,4,6-TCP. Histopathologic examination of the brain (cerebrum and cerebellum) of rats and mice exposed repeatedly to 2,4,6-TCP at oral doses as high as 720 and 1,356 mg/kg/day, respectively, revealed no treatment-related effects (Bercz et al. 1990; NCI 1979).

2,3,4,5-TeCP. When 20 Sprague-Dawley rats were exposed by unoccluded dermal application of 2,000 mg/kg 2,3,4,5-TeCP, one rat died after exhibiting clinical signs including hyperactivity, neuromuscular weakness, and convulsions (Shen et al. 1983).

2,3,4,6-TeCP. In Wistar rats exposed to a single dose of up to 632 mg/kg 2,3,4,6-TeCP (Hattula et al. 1981), or repeatedly to 200 mg/kg/day 2,3,4,6-TeCP for 90 days, no histopathological effects in the brain were observed (EPA 1986). In a single-dose dermal study of 2,3,4,6-TeCP and other tetrachlorophenols in rats, clinical signs observed before death were hyperactivity, neuromuscular weakness, and convulsions; the dermal LD_{50} values for 2,3,4,6-TeCP were 468 mg/kg in males and 565 mg/kg in females (Shen et al. 1983).

2,3,5,6-TeCP. A single dermal application of 2,000 mg/kg 2,3,5,6-TeCP, which was lethal to 2 of 20 (male and female) Sprague-Dawley rats, resulted in the following clinical signs before death: hyperactivity, neuromuscular weakness, convulsions, and death (Shen et al. 1983).

Mechanisms. Limited data were located on the mechanism of phenol- or chlorophenol-induced convulsions. Inhibition of oxidative phosphorylation and cellular respiration (discussed further in Section 2.18) is one possible mechanism.

Phenol administration in cats facilitated effects on central synaptic transmission at both excitatory and inhibitory synapses (Banna and Jabbur 1970). The authors proposed that certain phenols increase the amount of neurotransmitter released during synaptic transmission, resulting in convulsions. After intraperitoneal injection of several chlorophenols, convulsions predominated in those mice receiving the 2- and 4-CP compounds (Farquharson et al. 1958). Because these compounds have pK values ≥8.65 and would not be in the ionic form at physiologic pH, the investigators attributed the observed effect to the chlorophenol rather than the ion.

2.16 REPRODUCTIVE

Studies of reproductive effects in humans exposed to chlorophenols are limited to assessments using urinary levels of di- or trichlorophenols to assess exposure. Urinary levels are not considered to be reliable biomarkers of chlorophenol exposure; in fact, as noted earlier, urinary 2,5-DCP is used as a biomarker for exposure to *p*-dichlorobenzene. In animals exposed to chlorophenols by oral administration, decreases in implantations, litter size, and/or live births per litter have been reported after intermediate-duration exposure to 4-CP (200 mg/kg/day) (BSRC 2011), 2,4-DCP (46 mg/kg/day) (Exon and Koller 1985; Exon et al. 1984), and 2,4,6-TCP (46 mg/kg/day) (Exon and Koller 1985). Adverse effects on the male reproductive system (including increases in the percentage of abnormal sperm and

decreased sperm motility) were observed after acute-duration exposure to 2,4-DCP in mice (Aydin et al. 2009).

Harley et al. (2019) observed an association between decreased age at menarche and prenatal concentration of 2,4-DCP in maternal urine (mean shift of -0.8 months, 95% CI 1.6–0.0) in 179 girls followed as part of a longitudinal birth cohort study in California (CHAMACOS). No association was observed between maternal or peripubertal 2,4-DCP concentration and thelarche or pubarche in this group of girls.

The association between urinary 2,5-DCP and premature puberty was evaluated in three cohort studies (Binder et al. 2018; Harley et al. 2019; and Wolff et al. 2015, 2017) and one cross-sectional study (Buttke et al. 2012). 2,5-DCP in children's urine (peripubertal) was associated with delayed pubarche in girls (mean shift +1.0 month, 95% CI 0.1–1.9) in the CHAMACOS birth study noted earlier (Harley et al. 2019). In a cohort of more than 1,000 girls from New York City, Cincinnati, and northern California, 2,5-DCP concentrations in urine collected at baseline (ages 6–8 years) were associated with earlier age at first breast development (adjusted age at first breast development was 103 months for the highest quintile of 2,5-DCP concentration versus 112 months for the lowest quintile; p-value for comparison: 0.006) (Wolff et al. 2015). A follow-up study of this cohort (Wolff et al. 2017) revealed an association between 2,5-DCP in urine at baseline and earlier age at menarche (adjusted hazard ratio 1.34, 95% CI 1.06–1.71 in the highest quintile). In contrast, a cross-sectional analysis of 440 adolescent girls ages 12–16 years who participated in the NHANES survey (2003–2008) found that age at menarche was not associated with urinary 2,4-DCP levels (Buttke et al. 2012). However, in the latter study, menarche occurred prior to exposure measurement (urine sampling) in some participants; thus, a temporal relationship between the two could not be evaluated in the study.

Binder et al. (2018) evaluated the association between breast development and urinary concentrations of 2,4- and 2,5-DCP in a randomly-selected subset of 200 girls participating in the Growth and Obesity Cohort (Santiago, Chile). There were no significant associations between 2,4- or 2,5-DCP concentrations in urine samples collected over two different visits (corresponding to Tanner 1 and Tanner 4 stages of pubertal development) and breast volume, fibroglandular volume of the breast, or breast density measured at the second visit (Binder et al. 2018).

No association was observed between urinary 2,4- or 2,5-DCP and gonadarche or pubarche among 159 boys in the CHAMACOS cohort (Harley et al. 2019).

A longitudinal study of chemical exposure and reproductive hormones was conducted in a sample of 143 healthy, premenopausal women recruited at a research center in New York state (Pollack et al. 2018). Each participant provided between three and five urine samples at key points over two menstrual cycles for chemical analysis (including 2,4-DCP, 2,5-DCP, 2,4,5-TCP, and 2,4,6-TCP). Blood samples were collected from participants at several phases of the ovulatory cycle: the early follicular phase, at ovulation, and mid-luteal phase in cycle 1 and at ovulation in cycle 2. Serum levels of estradiol, progesterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were measured. Urinary concentrations ranged between 0.03 and 38.6 ng/mL for 2,4-DCP; 0.1–803.5 ng/mL for 2,5-DCP; 0.04–2.8 ng/mL for 2,4,5-TCP; and 0.03–8.5 ng/mL for 2,4,6-TCP. More than 49% of samples showed 2,4,5-TCP levels below the limit of detection, so this compound was not included in the analyses. In linear mixed models analysis of log-transformed hormone levels examining single chemicals, urinary 2,4-DCP was positively associated with progesterone levels (β 0.14, 95% CI 0.06–0.21) and inversely associated with FSH (β -0.08, 95% CI -0.11 to -0.04) after adjustment for covariates. No association was seen between 2,4-DCP and estradiol or LH, or between urinary 2,5-DCP or 2,4,6-TCP and any reproductive hormone (Pollack et al. 2018). Limitations of this study include the small sample size and relatively low prevalence of detectable 2,4-DCP (>35% of samples were below the detection limit).

2-CP. In a single-generation reproductive toxicity study using Sprague-Dawley rats exposed to 2-CP via drinking water from weaning through mating and parturition, the only difference from control was a marginal decrease ($p<0.10$) in litter size at the highest dose (76 mg/kg/day); no effects were seen at lower exposures (Exon and Koller 1985). This study was limited by assessment of few endpoints (percent fertile, litter size, numbers of live fetuses, birth and weaning weights, and survival to weaning); in addition, the fetus, not the litter, was the unit of statistical analysis.

No treatment-related histopathology findings were noted in the testes, epididymides, ovaries, or uteri of Sprague-Dawley rats given 2-CP (up to 1,000 mg/kg/day) by gavage for 28 days (Hasegawa et al. 2005).

4-CP. Exposure of Sprague-Dawley rats to 4-CP by gavage at doses up to 300 mg/kg/day for 28 days did not result in treatment-related histopathology findings in the testes, epididymides, ovaries, or uteri (Hasegawa et al. 2005). In a screening-level reproductive/developmental toxicity study (BSRC 2011), rats exposed to doses up to 200 mg/kg/day showed no effects on sperm parameters, estrous cyclicity, copulation, fertility, or gestation length. The number of implantation sites was reduced at this dose (14.6 versus 15.8 in controls). Although the difference from control was not statistically significant, the

number of offspring delivered was also lower, and the number of live offspring was significantly decreased ($p<0.05$) at 200 mg/kg/day (BSRC 2011). At 1,000 mg/kg/day (a dose that was lethal to 5/12 rats during the 14-day premating period), reduced numbers of implantations, offspring, and live offspring were also noted (but the changes were not statistically significant). No reproductive effects were noted at 40 mg/kg/day.

2,4-DCP. The effects of 2,4-DCP exposure were assessed in a 2-generation study in Wistar-Hanover rats (Aoyama et al. 2005). Groups of 24 rats/sex/group were administered a diet containing 2,4-DCP at concentrations of 0, 500, 2,000, or 8,000 ppm, which corresponded to doses of 0, 33.4, 134, or 543 mg/kg/day for males and 0**,** 49.1, 194, or 768 mg/kg/day for females. The parental generation (P) was exposed for 10 weeks prior to mating and through the gestation and lactation periods, then sacrificed upon weaning of their offspring. Offspring of the P generation (F1) were exposed to 2,4-DCP from weaning through mating, gestation, and lactation and were sacrificed upon weaning of their offspring. Offspring of the F1 generation (F2) were sacrificed at weaning. A statistically significant decrease in the number of implantation sites per female was detected in high-dose F1 rats, but not in the parental generation or in F1 rats receiving lower doses. No treatment-related changes in estrous cycle length, incidence of normal estrous cycles, number of primordial ovarian follicles, mating index, fertility index, gestation index, gestation length, pup number, viability at birth, or sex ratio, or pup viability during lactation were observed in the P or F1 generations. In addition, no treatment-related changes were observed in serum hormones that affect the reproductive system (FSH, LH, prolactin, estradiol, and progesterone) in female rats (assessed in F1 rats only) or in sperm parameters (number of testicular or epididymal sperm, sperm motility, and sperm morphology) in P and F1 males.

A teratogenicity study in which pregnant rats were treated with 2,4-DCP by gavage on GDs 6–15 at doses that caused maternal deaths and decreased body weight gain showed neither postimplantation loss nor changes in the numbers of resorptions and viable fetuses (Rodwell et al. 1989). When female Sprague-Dawley rats received 2,4-DCP in drinking water at doses up to 46 mg/kg/day from weaning through mating and parturition (~13 weeks total), the only exposure-related effect was a marginal decrease ($p<0.10$) in litter size (Exon and Koller 1985). The study examined few endpoints (percent fertile, litter size, numbers of live fetuses, birth and weaning weights, and survival to weaning); in addition, the fetus, not the litter, was the unit of statistical analysis.

Aydin et al. (2009) reported significant effects on the male reproductive system, including increased necrotic cell counts in the seminiferous tubules, increased percent abnormal sperm (>3-fold increase in

percent abnormal), and decreased sperm motility, in BALB/c mice receiving 1,000 ppm 2,4-DCP in drinking water (~260 mg/kg/day) for 14 days. However, sperm from male CD-1 mice fed 500 mg/kg/day 2,4-DCP for 90 days in drinking water did not exhibit impaired ability to fertilize ova (Seyler et al. 1984). The 2-generation study in rats (Aoyama et al. 2005) reported no effects on sperm parameters in parental or F1 males receiving dietary doses up 543 mg/kg/day 2,4-DCP.

No reproductive organ pathology was observed in rats or mice of either sex fed up to 2,000 or 2,600 mg/kg/day 2,4-DCP, respectively, for 13 weeks (NTP 1989). Reproductive organ pathology was not observed in male rats fed 440 mg/kg/day, in female rats fed 250 mg/kg/day, in male mice fed 1,300 mg/kg/day, or in female mice fed 8,210 mg/kg/day 2,4-DCP for 2 years (NTP 1989).

2,4,5-TCP. Gavage treatment of rats with 2,4,5-TCP at doses up to 1,000 mg/kg/day for 98 days had no effect on the weights of the testes or ovaries (McCollister et al. 1961). No other data pertaining to reproductive effects of 2,4,5-TCP were located.

2,4,6-TCP. A marginal ($p<0.10$) decrease in litter size was reported at the highest dose (46 mg/kg/day) in a single-generation reproductive toxicity study of 2,4,6-TCP (Exon and Koller 1985). In this study, female Sprague-Dawley rats received 2,4,6-TCP in drinking water (0, 3, 30, or 300 ppm) beginning at weaning and extending through mating (with untreated males) and parturition for a total duration of ~13 weeks. This study was limited by evaluation of few endpoints (percent fertile, litter size, numbers of live fetuses, birth and weaning weights, and survival to weaning); in addition, the fetus, not the litter, was the unit of statistical analysis.

Blackburn et al. (1986) observed no reproductive toxicity in a cross-mating study of 2,4,6-TCP administered by gavage at doses up to 1,000 mg/kg/day. Male and female rats were exposed for 2 weeks prior to mating for up to 10 days with untreated animals. Female rats continued exposure throughout gestation. Despite the fact that exposure-related deaths occurred in both sexes at 1,000 mg/kg/day, exposure had no effects on breeding success, litter size, or litter survival regardless of the sex treated. In a study of male reproductive effects in the same publication, gavage doses up to 1,000 mg/kg/day 10 weeks prior to mating with untreated females did not influence copulatory behavior, sperm count, motility, or morphology, nor were there any changes in weights of the testes, prostate, or seminal vesicles (Blackburn et al. 1986).

In a subchronic toxicity study, no effects were observed on the weights of the testes or ovaries in rats treated by gavage with 2,4,6-TCP doses up to 720 mg/kg/day (Bercz et al. 1990). Chronic (2-year) dietary exposure to 2,4,6-TCP in the diet likewise did not result in histologic changes in the testes, prostates, uteri, or ovaries of rats receiving doses up to 500 mg/kg/day or mice receiving doses up to 1,356 mg/kg/day (NCI 1979).

2,3,4,6-TeCP. An exposure-related trend in percent preimplantation loss, suggesting an effect on the process of implantation or early postimplantation viability, was observed when pregnant rats were treated with 2,3,4,6-TeCP by gavage at doses up to 200 mg/kg/day on GDs 6–l 5 (EPA 1987a, 1987b). However, because the study was not designed to examine the preimplantation/ implantation phase of reproduction, this finding requires confirmation. No histopathological changes were observed in the testes, ovaries, or uterus and cervix of rats treated by gavage with 2,3,4,6-TeCP at doses up to 200 mg/kg/day for 90 days (EPA 1986).

Mechanisms. Induction of oxidative stress has been proposed as a mechanism for the reproductive effects of 2,4-DCP. Dai et al. (2021) observed increases in reactive oxygen species (measured using dichlorofluorescein fluorescent probe) and apoptosis (measured using annexin-V staining) in oocytes from mice given seven daily intraperitoneal injections of 2,4-DCP at doses of 36, 72, or 180 mg/kg/day. The study authors suggested that these findings played a role in the decrease in *in vitro* fertilization rates seen when oocytes from the mice treated at 180 mg/kg/day were incubated with sperm from untreated mice (Dai et al. 2021).

The potential for chlorophenols to perturb estrogen and androgen activities has been evaluated in *in vitro* studies (Harris et al. 2005; Holmes et al. 2019; Kim et al. 2005; Okada et al. 2005; Yu et al. 2019). In vitro testing of 2-CP, 2,4,-DCP, and 2,4,6-TCP for estrogenic and antiestrogenic activities in the ER α transactivation assay showed that 2,4,6-TCP showed antagonistic activity, but only at the highest concentration tested (10^{-5} M); the other compounds were inactive as agonists or antagonists (Yu et al. 2019). Based on the results of competitive ERα binding assays, 2-CP and 2,4,6-TCP (the only chlorophenols tested) were classified as slight binders (relative binding affinities of ≤0.00004% where estradiol is 100%) (Holmes et al. 2019). Several chlorophenols were evaluated for their potential to inhibit isolated estrogen sulfotransferase (Harris et al. 2005). Sulfonation of estrogen, which results in a pharmacologically inactive substance, is an important process in the attenuation of the steroid-hormone signal in endometrial, mammary, and testicular tissues. 2,3-, 2,4-, 2,5-, and 2,6-DCP were potent inhibitors of isolated estrogen sulfotransferase. Other chlorophenols, such as 3,4-and 3,5-DCP and 4-CP,

inhibited estrogen sulfotransferase, but with a lower relative potency. The authors suggested that chlorophenol-induced inhibition of estrogen sulfotransferase could lead to increased intracellular levels of estrogen and thereby potentially alter estrogen-mediated cellular functions.

The potential for 2,4-DCP to potentiate 5α-dihydrotestosterone (DHT) action, as assessed by cell proliferation, was evaluated in human prostate cancer cells (lines AR expressed 22v1 and PC3) (Kim et al. 2005). Co-administration of 10 nano-molar (nM) 2,4-DCP enhanced the androgenic activity of DHT by 1.6-fold in comparison to 10 nM DHT alone. Translocation of the androgen receptor complex to the nucleus was increased in the presence of 2,4-DCP, suggesting that 2,4-DCP has the potential to alter androgen-induced transcriptional activity.

Limited *in vitro* data suggest that 2,4,6-TCP could affect reproductive function by interfering with steroidogenesis. In human adrenocortical H295R cells incubated with 2,4,6-TCP (10^{-7}–10^{-5} M, or 0.1–10 μM), significant, concentration-related decreases in CYP17 mRNA levels were detected (by reverse transcription-polymerase chain reaction [RT-PCR]) at all concentrations (Yu et al. 2019). In another study using the same test system, 2,4,6-TCP concentrations ≥1.1 μM significantly decreased the expression of steroidogenic acute regulatory protein (*StAR*), CYP19 (aromatase), and 17β-hydroxysteroid dehydrogenase (*17βHSD4*) and a concentration of 3.4 μM decreased the expression of CY11A and 3β-hydroxysteroid dehydrogenase (*3βHSD2*) (Ma et al. 2011). Ma et al. (2011) also observed significant decreases in testosterone and estradiol concentrations at the highest exposure concentration (3.4 μM). Time-course experiments showed that decreases in cellular cAMP levels occurred at the same time as decreases in *StAR* mRNA and protein levels, suggesting that cAMP signaling was involved in the inhibition of steroidogenesis (Ma et al. 2011).

Chlorophenols could exert effects on the reproductive system by interfering in the metabolism of key hormones. Liu et al. (2020a) examined the ability of 14 chlorophenols to inhibit the activity of CYP3A4, a key enzyme in the catabolism of testosterone. Incubation of each of the chlorophenols (at a concentration of 100 uM) with testosterone (the probe substrate) resulted in significant reductions in CYP3A4 activity. The chlorophenols inducing the greatest reductions in CYP3A4 activity (residual activity <40% of control) were 2,3,4-TCP, 2,4,5-TCP, 3,4,5-TCP, 2,3,4,5-TeCP, and pentachlorophenol. The inhibition exerted by the remaining compounds (including the monochlorophenols, 2,4-DCP, 2,4,6-TCP, 2,3,4,6-TeCP, and 2,3,5,6-TeCP) was more modest (residual activities 40–70%).

2.17 DEVELOPMENTAL

An epidemiological study investigated low birth weight of small-for-gestational-age (SGA) infants whose mothers were occupationally exposed to chlorophenols (Seidler et al. 1999). The cohort consisted of 3,946 German women recruited during weeks 15–28 of pregnancy. Occupational exposures to chlorophenols and other chemicals were estimated for each mother based on a job-exposure-matrix and used to assign chemical exposure categories (low, moderate, high) to each subject. The adjusted OR for infants classified as SGA was elevated for subjects with moderate exposure to chlorophenols (OR 7.0; 95% CI 1.2–43.0), which was the highest exposure category reported for chlorophenols (data for the high exposure category for chlorophenols were not reported). The authors identified several potential limitations of the study, including potential exposure misclassification from the application of the job-exposure-matrix and recognized co-exposures to other chemicals (Seidler et al. 1999).

Other human studies used urinary chlorophenol concentrations to assess exposure. These studies are of uncertain utility for chlorophenol hazard identification, because chlorophenols in urine may result from metabolism after exposure to other compounds (e.g., chlorobenzenes or pesticides, such as lindane, 2,4-D, and 2,4,5-T) rather than exposure to chlorophenols themselves.

A pilot case-control study nested within a large birth cohort (LIFECODES, Boston, Massachusetts) was conducted to evaluate potential associations between maternal urinary dichlorophenols (2,4- and 2,5-DCP) and birth size categorized as small- or large-for-gestational-age (SGA or LGA) (Bommarito et al. 2021). The numbers of subjects in the pilot study were small (n=31 cases of average size, n=31 SGA, and n=28 LGA). Maternal urine samples were collected 3 times during pregnancy (median gestation weeks 11, 26, and 35). There were no associations between birth size and maternal 2,4- or 2,5-DCP concentrations in this study.

Philippat et al. (2012) evaluated relationships among birth outcomes (birth weight, length, and head circumference) and urinary phenols and phthalates in a nested case-control study of male genital malformations. The case-control was nested in two birth cohorts (the EDEN and PELAGIE cohorts in France), and cases consisted of male newborns with hypospadias or undescended testes at birth. For each case, 3 controls were matched by recruitment site and date and by gestational week at which maternal urine was collected, yielding 72 cases and 216 controls. Urine samples collected between 6 and 19 weeks of gestation (PELAGIE) or between 24 and 30 weeks of gestation (EDEN) were analyzed for phthalates and phenols including 2,4- and 2,5-DCP. Analyses of birth outcome data were adjusted for oversampling

of malformation cases. Concentrations of 2,4- and 2,5-DCP in maternal urine were associated with decreased birth weight, and 2,4-DCP levels were also associated with decreased head circumference (Philippat et al. 2012). The authors noted that concentrations of the two dichlorophenols were highly correlated, so it is difficult to discern effects attributable to each individual compound in this study.

A second study evaluating the association between maternal urinary chlorophenol concentrations (including 2,4-DCP, 2,5-DCP, 2,4,5-TCP, and 2,4,6-TCP) and birth outcomes in a cohort of 1,100 women reported associations between creatinine-adjusted 2,4,6-TCP concentration and birth weight and between head circumference in male newborns and between both dichlorophenols and head circumference in female newborns (Guo et al. 2016). However, in this study, urine samples were collected at parturition, so the temporal relationship between exposure and outcome is highly uncertain. In a follow-up study of a subset of 377 mother-child pairs in the cohort (Guo et al. 2019), children's weight, height, and head circumference were measured when the children were 3 years of age. Urinary chlorophenol concentrations (2,4- and 2,5-DCP; 2,4,5- and 2,4,6-TCP) in the samples collected from mothers at birth and from infants at age 3 years were used to assess exposure. The frequency of 2,4,5-TCP was low (30–53%), so it was not analyzed further. The concentration of 2,4,6-TCP in maternal urine was associated with lower weight, height, and BMI z-scores (-0.50, -0.49, and -0.49 adjusted differences in z-scores, respectively, for 10-fold increase in creatinine-adjusted concentration). The association was not affected by adjustment for childhood urinary chlorophenol concentrations. Stratification by sex showed that the associations were inverse (decreasing weight, height, and BMI with increasing 2,4,6-TCP concentration) in boys but positive (increasing effects with increasing concentrations) in girls. A significant positive association was seen between 2,5-DCP concentration in girls' urine samples and higher weight z-score; there was no association between weight or other metrics and childhood urinary chlorophenols among boys (Guo et al. 2019). The latter analysis used urine samples collected at the same time as weight measurement, so the temporal relationship is uncertain. No significant association was observed between 2,4-DCP in maternal or childhood urine and any anthropometric parameter at age 3 years.

Berger et al. (2021) evaluated associations between urinary levels of 2,4- and 2,5-DCP in maternal urine during pregnancy and childhood weight at age 5 years in a study of the CHAMACOS longitudinal birth cohort. Urine samples were collected from mothers twice during pregnancy, and children's height and weight were recorded at age 5 years in 309 mother-child pairs. No significant association was seen between 2,4- or 2,5-DCP in maternal urine and BMI z-score or overweight/obese status at age 5 years.

The same authors (Berger et al. 2020) assessed the relationship between maternal urinary concentrations of 2,4- and 2,5-DCP during pregnancy and asthma, aeroallergies, and lung function (measured as forced expiratory volume in one second or FEV_1) at age 7 years. A total of 319 mother-child pairs from the CHAMACOS birth cohort had complete biomarker and covariate data and were included in the analysis. The results showed a positive association between urinary 2,4-DCP and probability of asthma and poorer lung function. In contrast, there was an inverse association between 2,5-DCP and probability of asthma. Neither chlorophenol was associated with aeroallergies in the children.

2-CP. Groups of 6–13 female Sprague-Dawley rats receiving a single gavage dose of 333, 667, or 1,000 mg/kg 4-CP on GD 11 showed no adverse changes in litter sizes, perinatal loss, pup weight, or litter biomass (Kavlock 1990). The only treatment-related effect was a transient decrease in maternal body weight at 1,000 mg/kg. No significant changes in offspring body or liver weights were observed in rats treated with 2-CP in drinking water at doses up to 73 mg/kg/day throughout gestation and lactation and for an additional 15 weeks (Exon and Koller 1982, 1983b, 1985).

When neonatal (PND 4) Sprague-Dawley rats were given 500 mg/kg/day 2-CP by gavage in a dose range-finding study, all animals died within 9 days (Hasegawa et al. 2005). In the main study, rats survived doses of 300 mg/kg/day for 18 days (PNDs 4–21). Transient decreases in body weight were noted (data not reported) at 300 mg/kg/day, but not at lower doses. There were no effects on developmental milestones (surface righting, visual reflexes, fur appearance, tooth eruption, eye opening, preputial separation, vaginal opening, and estrous cycle) at any dose up to 300 mg/kg/day in the main study (Hasegawa et al. 2005). Histopathology examinations of the rats treated with 300 mg/kg/day showed increased incidences of basophilic renal tubules in males (4/6 compared with 0/6 controls) and females (5/6 compared with 0/6 controls). This finding was not observed in the 50 mg/kg/day dose group and was not assessed in the 20 or 100 mg/kg/day 2-CP exposed groups (Hasegawa et al. 2005). No changes in weights or histopathology of the brain, pituitary gland, thymus, thyroid, heart, lungs, liver, spleen, adrenals, or reproductive organs were observed.

4-CP. All male and three of four female Sprague-Dawley rats given 500 mg/kg/day 4-CP by gavage beginning on PND 4 died (timing of deaths not reported) in a dose range-finding study, while there were no deaths at 300 mg/kg/day for 18 days in the main study (Hasegawa et al. 2005). The main study showed no treatment-related changes in body weights, developmental milestones (surface righting, visual reflexes, fur appearance, tooth eruption, eye opening, preputial separation, vaginal opening, and estrous cycle), or weights or histology of the brain, pituitary gland, thymus, thyroid, heart, lungs, liver, spleen,

adrenals, or reproductive organs (Hasegawa et al. 2005). No adverse treatment-related effects on offspring body weights, postnatal viability until PND 4, frequency of external anomalies, or necropsy findings were noted in a screening-level reproductive/developmental toxicity study of rats given gavage doses of 40 or 200 mg/kg/day before and during gestation; however, as discussed in Section 2.16, significantly fewer live offspring were delivered in the 200 mg/kg/day group (BSRC 2011).

2,4-DCP. Oral exposure of pregnant rats to 750 mg/kg/day 2,4-DCP for 10 gestation days induced a slight decrease in fetal weight and a statistically significant delayed ossification of sternal and vertebral arches and led to a slight insignificant increase in early embryonic deaths (0.8/average litter controls; 1.2/litter 750 mg/kg/day) (Rodwell et al. 1989). Maternal death occurred at this dose level, indicating that 2,4-DCP was not selectively toxic to embryos or fetuses. The authors indicated that, although the number of deaths and fetal weights differed from that of the concurrent controls, values were not different from the historical control data from their laboratory. No evidence of malformations in the offspring was found in this study. At 375 mg/kg/day, maternal body weight was reduced, and at ≥200 mg/kg/day, there was a decrease in maternal body weight gain. No effect on birth or weaning weight or survival to weaning was observed when female Sprague-Dawley rats received 2,4-DCP in drinking water at doses up to 46 mg/kg/day from weaning through mating and parturition (~13 weeks total) (Exon and Koller 1985). The study examined few endpoints, and the fetus, not the litter, was the unit of statistical analysis.

In a two-generation reproductive and developmental toxicity study, Wistar-Hanover rats (24/sex/dose) were exposed to 2,4-DCP in the diet for 10 weeks prior to mating and through mating, gestation, and lactation. Dietary concentrations of 0, 400, 2,000, and 8,000 ppm were estimated to yield oral doses of 0, 33.4, 134, or 543 mg/kg/day for males and 0, 49.1, 194, or 768 mg/kg/day for females. The percentage of pups with eyes open on lactation day 14 was significantly decreased in high-dose F1 and F2 pups compared to their respective controls. In F1 male pups, age at preputial separation was significantly increased at the high dose, but the delay was attributed to reduced body weight in this group. In contrast, F1 female pup vaginal opening was accelerated at the high dose despite a significant decrease in body weight in this group. In addition, uterine weights were significantly elevated in high-dose F1 and F2 weanlings (42 and 20%, respectively, compared with controls). Body weight gain and feed consumption were significantly decreased in high-dose F1 generation males and females throughout exposure (Aoyama et al. 2005). A slight but statistically significant decrease in the number of implantation sites in F1 parental females was observed; a small, nonsignificant decrease in implantation sites was also noted in F0 parental females (Aoyama et al. 2005). In F2 weanling females, microscopic examination of the uteri

showed increases in epithelial cell height in 7/10 females in the high-dose group (compared to 1/10 female controls).

2,4,5-TCP. Gavage administration of 650 mg/kg/day 2,4,5-TCP during organogenesis (GDs 6–15) produced no fetotoxicity, malformations, or structural terata in the offspring of Sprague-Dawley rats (Chernoff et al. 1990). Treatment resulted in maternal lethality (12 versus 0% in controls) and decrements in maternal weight gain (5–15 g less than controls) (Chernoff et al. 1990). In another developmental study, groups of mice received either a single gavage dose of 800–900 mg/kg 2,4,5-TCP on 1 day of gestation (any of GDs 8–15), or 250–300 mg/kg/day on any 3 days of gestation (GDs 7–9, 10–12, or 13–15) (Hood et al. 1979). A significant increase in the incidence of prenatal mortalities and resorptions was seen in dams dosed on day 14 with 800–900 mg/kg/day, but not in dams dosed on days 13–15 at 250–300 mg/kg/day. 2,4,5-TCP administered on other gestation days had no effect on resorption incidence or pup survival. 2,4,5-TCP administration did not affect mean fetal weight or the incidence of gross malformations, skeletal malformations, or cleft palates (Hood et al. 1979).

2,4,6-TCP. In a study designed to examine reproductive effects, a 10–11% decrease in litter weights was observed in litters of female rats treated by gavage with 2,4,6-TCP at 500 mg/kg/day for 2 weeks before mating and throughout gestation (Blackburn et al. 1986). No effects on litter weights were observed at 100 mg/kg/day, and no effects on survival to PND 42 were observed. No effects on body weight were observed among offspring of male rats treated by gavage with 2,4,6-TCP at 1,000 mg/kg/day for 10 weeks before mating (Blackburn et al. 1986). Because comprehensive examinations of offspring were not completed, this study is not sufficient to conclude that developmental effects do not occur following exposure to 2,4,6-TCP.

Maternal exposure of rats to 500 mg/kg/day 2,4,6-TCP produced a transient reduction in the body weight of offspring (Blackburn et al. 1986). No developmental effects were noted in the offspring of female rats exposed to 2,4,6-TCP throughout gestation (Blackburn et al. 1986; Exon and Koller 1985). In addition, no developmental effects were noted in the offspring of male rats treated with 2,4,6-TCP and untreated females (Blackburn et al. 1986). These studies were limited by the lack of reporting on the number of animals from which group means were calculated (Blackburn et al. 1986) and by a lack of reporting on maternal toxicity (Exon and Koller 1985).

2,3,4,6-TeCP. In a developmental study in which female Sprague-Dawley rats orally received purified 2,3,4,6-TeCP throughout organogenesis, the only effect on the fetus was delayed ossification of the skull

bones (Schwetz et al. 1974). The reported incidences were 14/173 (8%) and 18/104 (17%) at 0 and 30 mg/kg/day, respectively. When analyzed by litter, no statistical difference for delayed ossification was observed. Therefore, 30 mg/kg/day 2,3,4,6-TeCP is considered a NOAEL for developmental effects in rats. In a follow-up study, pregnant CD rats received 0,25, 100, or 200 mg/kg/day, in olive oil, every day during organogenesis (EPA 1987a, 1987b). Administration of the two highest doses resulted in decreases in corrected maternal body weight gain (dam body weight-gravid uterus weight) of 13 and 26%, respectively, with no effects at 25 mg/kg/day. Measurement of food intake indicated that these effects were not related to decreased food consumption. Minor variations between dose groups in fetal malformation and aberrations were not dose related. The investigators also noted a dose-related trend for 2,3,4,6-TeCP-mediated effects on implantation or postimplantation viability. No further evidence of maternal or fetotoxic effects were observed (EPA 1987a, 1987b). Based on maternal toxicity, this study identifies 100 mg/kg/day as a LOAEL and 25 mg/kg/day as a NOAEL for developmental effects.

Mechanisms. Few data on mechanisms of developmental toxicity for chlorophenols are available. Kelley et al. (2019) reported a positive correlation between urinary levels of 2,4- and 2,5-DCP collected from 56 pregnant women during early pregnancy and IL-8 levels in plasma during the first trimester. However, no association was observed when covariates were considered in linear regression analysis. In a similar study, 2,5-DCP was determined to be a primary driver of the positive association observed between urinary phenol levels and oxylipins in the plasma of 90 pregnant women (Welch et al. 2021). Oxylipins are bioactive lipids that are involved in the regulation of inflammatory responses. Liu et al. (2021) evaluated the effects of three trihalophenols (2,4,6-TCP, tribromophenol, and triiodophenol) on human extended pluripotent stem cells *in vitro.* In this test system, 2,4,6-TCP was cytotoxic at a concentration of 200 μM. At lower concentrations of 10 and 50 μM, 2,4,6-TCP was shown to inhibit expression of key pluripotent marker genes (OCT4 and SOX2) and to modify cell differentiation by inhibiting transcription of endodermal (FOXA2, SOX17) and mesodermal (BRACHYURY and α-SMA) marker genes while increasing transcription of ectodermal (β-tubulin and nestin) marker genes (measured using quantitative reverse transcription polymerase chain reaction [RT-qPCR]) (Liu et al. 2021).

2.18 OTHER NONCANCER

Three studies used NHANES data to examine the relationship between urinary levels of dichlorophenols and obesity among children aged 6–19 years (n=6,770 and 2,372; Twum and Wei 2011 and Wu et al. 2020, respectively) or among adults aged 20–85 years (n=2,963; Wei et al. 2014). In all three studies, urinary 2,5-DCP concentrations were associated with higher prevalence of obesity, while 2,4-DCP was

not. Wu et al. (2020) also evaluated BMI in children and observed no association with 2,4- or 2,5-DCP in urine. Wei and Zhu (2016a, 2016b) observed concentration-related associations between 2,5-DCP in urine and higher prevalences of diabetes and metabolic syndrome in adults; no associations were seen with urinary levels of 2,4-DCP in either study. In a cross-sectional study conducted in Norway, Vindenes et al. (2021) reported an inverse association between urinary 2,4-DCP and BMI in 496 adults (change in BMI of -0.02, 95% CI -0.03, -0.01; p=0.007). Urinary levels of 2-5-DCP were not associated with BMI (Vindenes et al. 2021).

Mechanisms of Toxicity. Chlorophenols have been shown to uncouple mitochondrial oxidative phosphorylation (Cascorbi and Ahlers 1989; Farquharson et al. 1958; Hallinger et al. 2020; Izushi et al. 1988; Mitsuda et al. 1963; Narasimhan et al. 1992; Ravanel et al. 1985, 1989; Shannon et al. 1991; Stockdale and Selwyn 1971; Weinbach and Garbus 1965). During the Krebs cycle, lipophilic weak acids uncouple oxidative phosphorylation from electron transport by picking up protons, diffusing across the inner mitochondrial membrane, deprotonating, and returning to pick up more protons, thereby dissipating the pH gradient and membrane electrochemical potential needed for the formation of ATP (Lou et al. 2007; Stryer 1988). During this uncoupling, electron transport from NADH to oxygen can increase several-fold, but the energy produced, which is normally stored as the chemical potential of ATP, is released as heat. Severe toxic manifestations of the uncoupling of oxidative phosphorylation may include central nervous system depression followed by increased respiration, hyperthermia, blood pressure rise, progressive neuromuscular weakness, convulsions, muscle rigidity, and death.

Most of the data on chlorophenol-induced uncoupling have been from *in vitro* mitochondrial preparations, but one study demonstrated the metabolic effects (such as increased body temperature and dyspnea) in male rats exposed *in vivo* (Farquharson et al. 1958). In this study, the manifestations of uncoupling increased with increasing chlorination and decreasing pK, as shown in the Table 2-11.

Table 2-11. Relationship Between Degree of Chlorination and Symptoms of Uncoupling in Rats Exposed by Intraperitoneal Injection

Compound	pK	LD_{50}	Convulsions	Change in rectal temperature
Phenol	9.98	250	+	-2.5
4-CP	9.37	281	+	-2.5
2-CP	8.65	230	+	-2.0
2,4-DCP	7.85	430	Twitching	-0.5
2,4,5-TCP[a]	7.07	355	–	+0.5
2,4,6-TCP[a]	6.62	276	+	+0.5

Table 2-11. Relationship Between Degree of Chlorination and Symptoms of Uncoupling in Rats Exposed by Intraperitoneal Injection

Compound	pK	LD_{50}	Convulsions	Change in rectal temperature
2,3,4,6-TeCP[a]	5.46	130	–	+4.0

[a]Rigor mortis within 5 minutes of death.

Source: Farquharson et al. 1958

The results of a number of *in vitro* studies (Cascorbi and Ahlers 1989; Izushi et al. 1988; Mitsuda et al. 1963; Narasimhan et al. 1992; Shannon et al. 1991; Stockdale and Selwyn 1971) indicate a concentration-dependent, triphasic effect of chlorophenols on phosphorylation and cellular respiration. At low concentrations, uncoupling produces stimulation of state 4 (resting state) respiration as a result of increased adenosine triphosphatase (ATPase) activity in the absence of a phosphate acceptor. Inhibition of state 3 (active) respiration is also observed. At moderate concentrations, resting respiration is neither stimulated nor inhibited. Significant inhibition of respiration, associated with a breakdown of the electron transport process and decreased ATPase activity, occurs at very high concentrations. These concentrations are also associated with mitochondrial swelling and disruption of the mitochondrial matrix structure. Investigators have cited two independent mechanisms to explain these effects on cellular metabolism. Uncoupling activity has been attributed to a protonophoric effect (a disruption of the energy gradient across the mitochondrial membrane resulting from distribution of chlorophenols in the phospholipid bilayer of the membrane), whereas inhibition of cellular respiration has been attributed to a direct action on intracellular proteins.

The results of these and other studies also illustrate that higher order chlorophenols have the greatest effects on cellular metabolism. Hallinger et al. (2020) observed concentration-dependent uncoupling by 2,4,5-TCP in an *in vitro* respirometric screening assay in Hep2G cells. In this assay 2,4,6-TCP exhibited weak uncoupling, while 2,4-DCP was inactive (Hallinger et al. 2020). Older studies showed that 2-CP and 4-CP are <7% as potent as tetrachlorophenol in uncoupling oxidative phosphorylation and inhibiting cellular respiration (Cascorbi and Ahlers 1989; Janik and Wolf 1992; Narasimhan et al. 1992; Weinbach and Garbus 1965). Within the chlorophenol series, two physicochemical parameters, the a-Hammett constant, a measure of electron withdrawing ability, and the octanol-water partition coefficient (log K_{ow}), accounted for 98% of the variability in the inhibition of ATPase activity (Cascorbi and Ahlers 1989).

A repeated-measures study of the relationship between urinary contaminants and plasma antioxidant enzyme levels (erythrocyte glutathione peroxidase, glutathione reductase, plasma glutathione peroxidase,

or superoxide dismutase) in 143 healthy women between 18 and 44 years old showed an inverse association between the urine concentration of 2,5-DCP and plasma SOD (Pollack et al. 2020). In contrast, urinary 2,4,6-TCP was associated with increased plasma glutathione peroxidase and glutathione reductase levels. No significant associations were seen between 2,4-DCP or 2,4,5-TCP in urine and plasma antioxidants (Pollack et al. 2020). In a study of 54 pregnant women (participants in the Puerto Rico Testsite for Exploring Contamination Threats, or PROTECT), urinary markers of oxidative stress (OHdG and isoprostane) were not correlated with urinary concentrations of 2,4-DCP or 2,5-DCP (Watkins et al. 2015). *In vitro* data suggest that chlorophenols may induce oxidative stress through the formation of reactive metabolites (Bukowska et al. 2003, 2004, 2016; Truffin et al. 2003). Results of an *in vitro* study in human hepatoma cells indicate that reactive metabolites of 4-CP may induce or contribute to conditions of oxidative stress (Truffin et al. 2003). Incubation of hepatoma cells (Hep G2 cell line) with 350 μM 4-CP for 24–48 hours significantly reduced the activities of cytochrome P-450 reductase, catalase, and glutathione peroxidase as well as levels of glutathione and ATP. In addition, mRNA expression of cytochrome P-450 isozymes, CYP3A7 and CYP2E1, was significantly increased, with more pronounced effects on CYP3A7. Incubation of human peripheral blood mononuclear cells with relatively high concentrations of 2,4-DCP resulted in significant increases in oxidative damage measured as 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) oxidation, lipid peroxidation, and protein carbonylation (Bukowska et al. 2016). *In vitro* exposure of human erythrocytes to 2,4-DCP (Bukowska et al. 2003) and 2,4,5-TCP (Bukowska et al. 2004) resulted in decreased levels of glutathione and antioxidant enzyme (SOD, catalase) activities, which are indicative of changes associated with oxidative stress. There were no changes observed for total glutathione levels (reduced plus oxidized glutathione) or glutathione reductase activity when cells were exposed to 100 ppm 2,4,5-TCP *in vitro*. Results of these studies are consistent with oxidative stress potentially induced by chlorophenol-derived free radicals.

Comparative cytotoxic effects and mediation of cell death through induction of apoptosis were evaluated for 4-CP, 2,4-DCP, 2,3,4-TCP, and pentachlorophenol in fibroblast L929 cells (mouse connective tissue fibroblast cell line) (Chen et al. 2004). Incubation of L929 cells with each of these compounds induced significant dose-and time-dependent reductions in cell growth. The results of deoxyribonucleic acid (DNA) fragmentation analysis (for 4-CP, 2,4-DCP, and 2,3,4-TCP), which is a distinctive feature of apoptosis, revealed dose-and time-dependent effects for these chlorophenol exposures. Observations are consistent with induction of cell death through apoptosis as the mechanism of action for exposure to 4-CP, 2,4-DCP, or 2,3,4-TCP, as opposed to cell necrosis for pentachlorophenol (Chen et al. 2004).

2.19 CANCER

Several case-control studies and an ecological study have suggested possible links between chlorophenol exposure and NHL, soft tissue sarcoma, and nasal cancers (Lampi et al. 2008). In the case-control studies (Garabedian et al. 1999; Hoppin et al. 1998; Mirabelli et al. 2000; Richardson et al. 2008), exposure to individual chlorophenols was not evaluated; rather, exposure to chlorophenols as a class was assessed based on job history, and the subjects may have been exposed to pentachlorophenol. In addition, in the ecological study (Lampi et al. 2008), the water supply to which the community was exposed was contaminated with pentachlorophenol in addition to 2,4,6-TCP and 2,3,4,6-TeCP. As a result, it is not possible to determine whether the observed associations might be attributable to exposure to one or more of the chlorophenols addressed in this profile, or to pentachlorophenol exposure.

A retrospective cohort study was conducted by Demers et al. (2006) evaluating the association between pentachlorophenol and 2,3,4,6-TeCP, and cancer morbidity and mortality, in sawmill workers in British Columbia, Canada. The cohort consisted of 27,464 former male workers who were employed at 14 different sawmills during the period from 1950 to 1995. Cancers that occurred during the period from 1969 to 1995 were identified from records in cancer registries. No clear evidence was found to link 2,3,4,6-TeCP exposure to cancer mortality or incidence. The authors noted that the use of tetrachlorophenol at the sawmill was more recent than pentachlorophenol, and that the follow-up time for tetrachlorophenol may thus have been inadequate to evaluate its association with cancer.

A large population-based, case-control study yielded data on the association between occupational exposures to chlorophenols and three cancer types: soft tissue sarcoma (Hoppin et al. 1998), NHL (Garabedian et al. 1999), and nasal or nasopharyngeal cancers (Mirabelli et al. 2000). Cases consisted of men born between 1929 and 1953 whose cancers were reported to one of eight cancer registries in the United States between 1984 and 1988. The same group of 1,909 controls was used for all three cancers. Job history information was obtained via telephone interviews of cases and controls and each job was classified by chlorophenol exposure (unexposed, minimal exposure, moderate exposure, and substantial exposure) by an industrial hygienist based on exposure intensity and level of confidence with exposure intensity assignment. For NHL, adjusted ORs were based on 995 cases and 1,783 controls (Garabedian et al. 1999). The adjusted OR for “ever being occupationally exposed to low, medium, or high concentrations of chlorophenols with medium or high confidence levels” was 1.07 (95% CI 0.93–1.24; 255 cases, 399 controls), and when exposure durations were restricted to >8 years, the OR increased to 1.51 (95% CI 0.88 to 2.59; 18 cases, 8 controls).

For soft-tissue sarcoma, adjusted ORs were based on a total of 295 cases and 1,908 controls (Hoppin et al. 1998). The risk of soft tissue sarcoma increased with exposure duration, especially in those classified as having substantial exposure. The OR among those with at least 10 years of exposure to chlorophenols was 7.8 (95% CI 2.46–24.65) (Hoppin et al. 1998). However, there was no increase in OR with exposure intensity or confidence in exposure intensity. For nasopharyngeal cancer, there were 43 nasal carcinoma cases, 92 nasopharyngeal carcinomas cases, and 1,909 controls. Mirabelli et al. (2000) found an increased risk of nasopharyngeal cancers for workers placed in the medium chlorophenols exposure group (adjusted OR 1.94; 95% CI 1.03–3.50; 18 exposed cases; 244 controls) and the high exposed group (OR 2.64; 95% CI 1.11–5.78). In addition, risk of nasopharyngeal cancer increased with increasing exposure duration (OR for exposure >10 years 9.07; 95% CI 1.41–42.9; 3 exposed cases; 7 controls) (Mirabelli et al. 2000). Several limitations preclude drawing definitive conclusions from these studies, including: (1) potential misclassification of exposure from use of a post-hoc categorical assignment of subjects to exposure categories, rather than specific measurements of exposure history (e.g., workplace or biomarker monitoring); (2) possibly previous or concurrent chemical exposures, which may have contributed to the outcomes that were not adjusted for in the study design or data analysis (e.g., solvents, formaldehyde, chromium, nickel, pentachlorophenol, and chlorinated dibenzo-*p*-dioxins and dibenzofurans); and (3) lack of evidence of dose-response relationships in some studies.

A potential association between chlorophenol exposure and NHL was reported in a case-control study of NHL in northern Germany (Richardson et al. 2008). A total of 858 incident cases of NHL diagnosed between 1986 and 1998 were compared with 1,821 age, sex, and region-matched population controls. Subjects were interviewed for detailed occupational histories and exposures were estimated with a job-exposure matrix. An increased risk for high malignancy NHL was reported (OR 1.95, 95% CI 1.32–2.87); however, there was no exposure-response trend when analyses were performed by tertile of cumulative chlorophenol exposure.

A study was conducted in Southern Finland to determine if drinking water contaminated with chlorophenols was associated with cancer morbidity (Lampi et al. 2008). At the end of 1987, environmental sampling of groundwater near a village where 2,000 residents lived revealed chlorophenols levels ranging from 70 to 140 μg/L. The residents used the groundwater as a source of drinking water. The village was located near a sawmill that used fungicides containing chlorophenols (primarily 2,3,4,6-TeCP); the fungicides also contained pentachlorophenol, 2,4,6-TCP, and polychlorinated dioxin and furan impurities. Polychlorinated dioxins and furans were not detected during groundwater

monitoring. Environmental sampling of the deep aquifer in the vicinity of the sawmill revealed chlorophenols ranging from 56,000 to 190,000 μg/L; chlorophenols were also detected in the fish and water from a local lake. In 1987, the municipal drinking water intakes from groundwater near the area were closed. Evaluation of the cancer incidences in the village during three periods (1953–1971, prior to exposure; 1972–1986, during exposure; and 1987–2006, after exposure ended) showed higher incidences of soft tissue cancers and NHL (compared with incidence rates for the region where the village was located) in the period during exposure. In that period, the standardized incidence ratios (SIRs) were 3.19 (95% CI 1.17–6.95) for soft tissue cancer and 2.08 (95% CI of 1.14–3.49) for NHL. No increase in the incidences of colon cancer, bladder cancer, Hodgkin's lymphoma, or leukemia was observed during the exposure period. The soft tissue cancer and NHL incidence rates did not differ from the reference rates during the periods before or after exposure, suggesting an association with the chlorophenol exposure.

Several other epidemiological studies (Eriksson et al. 1981, 1990; Hardell and Eriksson 1988; Hardell et al. 1981, 1995; Hooiveld et al. 1998; Kogevinas et al. 1997; Lynge 1985; Saracci et al. 1991; Zendehel et al. 2014) have examined potential associations between cancer and occupational exposure to chlorophenols during the manufacture or use of phenoxy herbicides (e.g., 2,4-D [2,4-dichlorophenoxy-acetic acid], 2,4,5-T [2,4,5-trichlorophenoxyacetic acid], Agent Orange [mixture of 2,4-D and 2,4,5-T], and related compounds). In these settings, workers may have been exposed to pentachlorophenol, phenoxy herbicide compounds, and polychlorinated dioxin and furan contaminants in addition to chlorophenols that are the subject of this profile. Although the studies suggest associations between some cancer types and these workplace exposures, most of the studies that focused narrowly on chlorophenol exposure (other than pentachlorophenol) have not shown any association.

IARC coordinated an international collaborative analysis of workers exposed to phenoxy herbicides and related chlorophenols and dioxin contaminants. The most recent publication on this effort included 21,863 male and female workers across 36 cohorts and 12 countries who were followed from 1939 to 1992 (Kogevinas et al. 1997). Among the workers who were exposed to phenoxy herbicides and/or chlorophenols but not exposed to TCDD or higher chlorinated dioxins, an elevated standardized mortality ratio (SMR) of 6.38 (95% CI 1.32–18.65) was reported for adrenal gland tumors. SMRs for NHL and lung cancer were close to unity. There was a slight increase in the SMR for soft tissue sarcoma (SMR 1.35), but this was based on only two deaths. Other tumor types for which some evidence of association was observed in this subgroup include sinonasal tumors (SMR 3.8) and thyroid tumors

(SMR 2.17). None of the SMRs showed relationship with years since first exposure or duration of exposure.

In a cohort of 549 male Dutch chemical factory workers exposed to phenoxy herbicides, chlorophenols, and polychlorinated dioxins and furans between 1955 and 1991, increased SMRs were observed for cancers of the bladder, kidney, and urinary organs (Hooiveld et al. 1998). When compared with an internal comparison group of 482 unexposed male workers, the relative risks (adjusted for age, calendar year at end of follow up, and time since first exposure) for cancers of the urinary organs and respiratory tract were elevated (relative risks [RRs] in the range of 4.2–7.5) but CIs included 1.0. The adjusted RR for NHL was 1.7 based on only one unexposed and three exposed deaths.

A number of case-control studies have reported associations between exposure to phenoxy herbicides, chlorophenols, and/or dioxins and NHL or soft tissue sarcomas in Sweden (Eriksson et al. 1981, 1990; Hardell and Eriksson 1988; Hardell et al. 1981). In a meta-analysis of soft tissue sarcomas in these studies, Hardell et al. (1995) reported an increased odds ratio for exposure specifically to chlorophenols. However, the authors indicated that pentachlorophenol was the primary chlorophenol used in Sweden at the time of the exposures, and indeed most of the subjects in the group exposed to chlorophenols were exposed to pentachlorophenol (27/34 cases and 30/34 controls).

Zendehel et al. (2014) conducted a meta-analysis of five studies evaluating lung cancer mortality among pesticide production workers exposed to phenoxyacetic acids and chlorophenols. In the three studies examining groups exposed only to chlorophenols (no TCDD or phenoxyacid exposures), there was no association with lung cancer. In a retrospective cohort study on Danish phenoxy herbicide workers, there were no cases of soft tissue sarcoma or malignant lymphoma among subjects (n=615) in the factory manufacturing only 2,4-DCP and 4-chloro-*o*-tolyloxy-acetic acid (MCPA) (Lynge 1985). Other factories evaluated by this author manufactured a wide range of phenoxy herbicides in addition to 2,4-DCP. Similarly, no deaths from soft tissue sarcoma or NHL were reported among workers producing or spraying exclusively chlorophenols in a cancer mortality study (2,377 deaths among a population of 18,910) of sprayers and production workers exposed to chlorophenoxy herbicides and chlorophenols (Saracci et al. 1991).

In well-conducted chronic cancer bioassays of chlorophenol compounds, 2,4-DCP did not induce an increase in cancer incidence in rats and mice treated with 2,4-DCP in the diet at doses up to 440 mg/kg/day (rats) and 1,300 mg/kg/day (mice) (NTP 1989), while rats and mice exposed to 2,4,6-TCP

in the diet exhibited increased incidences of leukemia and liver cancer (respectively) (NCI 1979). Other chlorophenols discussed in this profile have not been adequately tested for potential carcinogenicity.

2-CP. In an oral carcinogenicity study located, groups of Sprague-Dawley rats received prenatal, postnatal, or both pre- and postnatal exposure to 2-CP (Exon and Koller 1985). The exposure concentrations were 0, 5, 50, and 500 ppm in drinking water (0, 0.62, 6.2, or 62 mg/kg/day). Under all exposure conditions, 2-CP administration had no effect on the incidence, latency, or types of tumors relative to the untreated controls. Additional groups of gravid dams received ethylurea and nitrite, precursors of the carcinogenic initiator ethylnitrosourea (ENU), on GDs 14 and 21. No consistent effects on either tumor incidence or latency occurred in rats treated with ENU and then treated either prenatally or postnatally with 2-CP. The groups of males receiving ENU and both prenatal and postnatal 2-CP had increased tumor incidence and decreased tumor latency relative to a control group receiving ENU only. The investigators indicated that the combined changes were marginally statistically significant ($p=0.10$) in comparison to a group receiving the initiator ENU only. ENU-exposed female rats also exposed pre- and postnatally to 2-CP showed no consistent, concentration-related effects on either tumor incidence or latency (Exon and Koller 1985). Findings in the combined-exposure male treatment groups indicate that 2-CP may be either a cocarcinogen or a tumor promotor. However, an analysis of incidence and latency data suggests that the effects may not be concentration related. No effects on tumorigenicity were found in similar studies with 2,4-DCP given in drinking water at 0.62, 6.2, or 62 mg/kg/day. It is not clear whether a maximum tolerated dose was achieved in these studies (Exon and Koller 1985).

In 15-week mouse initiation-promotion studies, 2-CP showed tumor promoting activity (Boutwell and Bosch 1959); however, the significance of these results is limited by the lack of appropriate vehicle control groups, irritation, and the reporting of only gross pathological effects (EPA 1980). One application of the known tumor initiator, 9,10-dimethyl-1,2-benzanthracene (DMBA), to the middorsal region of mice was followed by twice weekly dermal applications of 25 μL of a 20% solution of 2-CP. Compared to DMBA treatment alone, 2-CP increased the number of skin tumors (Boutwell and Bosch 1959). In a study in which no initiator was used, 2-CP applied to the backs of mice twice per week for 12 weeks resulted in papillomas in 46% of the mice (Boutwell and Bosch 1959). No carcinomas were observed.

2,4-DCP. Chronic carcinogenicity bioassays in rats and mice treated with 2,4-DCP in the diet at doses up to 440 mg/kg/day for rats and 1,300 mg/kg/day for mice did not provide any evidence that 2,4-DCP is carcinogenic (NTP 1989).

2,4-DCP exhibited tumor promoting activity in a 15-week mouse dermal initiation-promotion study in which 2,4-DCP was applied twice weekly (25 μL of a 20% solution) after a single application of DMBA (Boutwell and Bosch 1959). An increase in the number of skin tumors was seen after promotion with 2,4-DCP compared with DMBA treatment alone.

2,4,6-TCP. Carcinogenicity bioassays with rats and mice have shown increased incidences of leukemia and liver cancer with chronic oral exposure to 2,4,6-TCP (NCI 1979). In male rats, chronic oral exposure to 2,4,6-TCP in the diet produced a significant dose-related increase in the incidence of monocytic leukemia (NCI 1979). The increase was statistically significant compared to both concurrent and historical control incidences. An increased incidence of leukemia also occurred in female rats; however, the increase was not significant compared to the controls. In addition, leukocytosis and monocytosis as well as hyperplasia of the bone marrow were induced in treated male and female rats that did not develop leukemia. In rats with leukemia, there were large numbers of circulating monocytes in the blood that ranged from well-differentiated monocytes to immature and blast forms. Monocytes were often observed in the liver, spleen, lymph tissue, and bone marrow and occasionally in the lungs, adrenals, and other organs.

In both male and female B6C3Fl mice treated chronically with 2,4,6-TCP in the diet, a significant dose-related increase in the incidence of hepatocellular adenomas and carcinomas was noted (NCI 1979). Statistically significant increases in liver tumor incidences were observed in both males and females when compared with both concurrent and historical control groups. Liver damage, including individual liver cell abnormalities, focal areas of cellular alteration, and focal and nodular areas of hyperplasia were commonly present in the treated mice. Significant limitations of this study included the failure to report the dioxin content of the 2,4,6-TCP formulation, changes in the dosing regimen of mice, and no testing of organ function.

A single oral dose of 2,4,6-TCP (200 mg/kg) did not significantly increase skin tumors in mice treated dermally with a tumor promoter (12-O-tetradecanoylphorbol-13-acetate [TPA]) relative to TPA alone, suggesting that 2,4,6-TCP does not act systemically as an initiator (Bull et al. 1986). Other studies also examined the possible carcinogenic effects of 2,4,6-TCP, but contained limitations that preclude a conclusion (Innes et al. 1969; NCI 1968; Stoner et al. 1986). The limitations included early termination of the experiment (24 weeks) (Stoner et al. 1986), only one treatment group (Innes et al. 1969; NCI 1968),

a small number of treated animals (Innes et al. 1969; NCI 1968), and a change in dosing regimen and method of exposure (Innes et al. 1969; NCI 1968).

Skin tumor initiation and promotion assays using 2,4,6-TCP have not shown evidence of dermal tumor initiation or promotion activity. 2,4,6-TCP did not have initiating activity in another study in which mice were treated with a dermal dose of 200 mg/kg/day 2,4,6-TCP followed 2 weeks later by 20 weeks (3 times/week) of dermal TPA treatment (Bull et al. 1986). In addition, 2,4,6-TCP did not increase the number of skin tumors when applied (25 μL of a 20% solution) twice weekly for 15 weeks to the skin of mice pretreated with a single dermal application of DMBA, when compared with the incidence in mice treated only with DMBA (Boutwell and Bosch 1959).

2.20 GENOTOXICITY

Available evidence indicates that the chlorophenols are not potent mutagens; however, there is evidence that they are capable of causing chromosomal aberrations and DNA damage. The lack of genotoxicity seen in most of the available *in vivo* studies may be attributable to rapid urinary excretion of chlorophenols in these single-dose studies (Borzelleca et al. 1985a; Kitchin and Brown 1988).

In a human study, Rocha et al. (2018) examined urinary concentrations of 2,4-DCP, 2,5-DCP, 2,4,5-TCP, and 2,4,6-TCP (and 36 other chemicals) in correlation with the oxidative DNA damage marker 8-hydroxy-2'-deoxyguanosine (8OHDG) in Brazilian children. Urinary levels of 2,4-DCP and 2,5-DCP (but not 2,4,5-TCP or 2,4,6-TCP) were correlated with higher levels of 8OHDG. No exposure information was reported; thus, it is not known whether the chlorophenols detected in the urine resulted from exposure to chlorophenols or metabolism of dichlorobenzene and/or other compounds.

Reactive intermediates produced by incubation of 2,4-DCP, 2,4,5-TCP, or 2,4,6-TCP with horseradish peroxidase formed covalent adducts with deoxyguanosine in isolated calf thymus DNA and in isolated deoxyguanosine (Dai et al. 2005).

Genotoxicity testing results for each chlorophenol are summarized below. Results of *in vitro* genetic testing are presented in Tables 2-12 (2-CP), 2-13 (4-CP), 2-14 (2,4-DCP), 2-15 (2,4-DCP), 2-16 (2,4,5-TCP), 2-17 (2,4,6-TCP), and 2-18 (other chlorophenols); *in vivo* genotoxicity test results are described in text for the corresponding chlorophenol.

2-CP. 2-CP has been tested in one *in vivo* and several *in vitro* genotoxicity assays (see Table 2-12). The results of prokaryotic mutagenicity (Ames) assays for 2-CP were negative with and without metabolic activation (Haworth et al. 1983; Rapson et al. 1980). Similarly, 2-CP did not induce DNA-repairing genes in an umu test system in *Salmonella typhimurium* (Ono et al. 1992), nor did it induce DNA damage in a prophage induction assay with *Escherichia coli* (DeMarini et al. 1990). In mammalian *in vitro* systems, 2-CP induced slight-to-moderate increases in c-mitosis (indicating disturbances of the spindle function) and a significant increase in aneuploidy in cultured Chinese hamster lung cells (Onfelt 1987). In human lymphocytes, 2-CP induced concentration-related increases in the frequencies of micronuclei in a cytokinesis block micronucleus assay (Vlastos et al. 2016); however, cytotoxicity (measured as a significant change in the cytokinesis block proliferation index [CBPI]) was seen at the same doses. 2-CP also induced double-stranded DNA breaks (measured using the γ-H2AX focus assay) in human gingival fibroblasts (Shehata et al. 2012).

In an *in vivo* study in ICR mice, gavage administration of up to 69 mg/kg/day 2-CP in corn oil for 14 days did not increase sister chromatid exchange (SCE) rates in testicular or bone marrow cells (Borzelleca et al. 1985a). Details on the time between dosing and evaluation were not provided by the authors.

Table 2-12. Genotoxicity of 2-Chlorophenol *In Vitro*

		Results		
		Activation		
Species (test system)	Endpoint	With	Without	Reference
Prokaryotic organisms:				
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Mutation	–	–	Haworth et al. 1983
S. typhimurium TA100	Mutation	NA	–	Rapson et al. 1980
S. typhimurium TA1535/pSK1002 (umu test)	DNA damage/repair	–	–	Ono et al. 1992
Escherichia coli WP2s(λ) (prophage induction)	DNA damage/repair	–	–	DeMarini et al. 1990
Eukaryotic organisms:				
Chinese hamster V79 cells	Chromosomal aberrations	NA	+	Onfelt 1987
Human lymphocytes (CBMN)	Micronuclei	NA	+	Vlastos et al. 2016
Human gingival fibroblasts (γ-H2AX)	DNA damage	NA	+	Shehata et al. 2012

+ = positive results; – = negative results; DNA = deoxyribonucleic acid; NA = not applicable

4-CP. *In vitro* genotoxicity data are available for 4-CP; no *in vivo* studies of genotoxicity were identified for 4-CP. In *S. typhimurium* reverse mutation assays, treatment with 4-CP generally did not produce an increased number of revertants in the presence or absence of metabolic activation (DeMarini et al. 1990; Haworth et al. 1983; Kubo et al. 2002; Rapson et al. 1980) (see Table 2-13). In one study, 4-CP had a marginally positive response in strain TA1537 (Seuferer et al. 1979). In another study (Strobel and Grummt 1987), 4-CP induced increased numbers of revertants in *S. typhimurium* strains TA97, TA98, TA100, and TA104 with the most pronounced effects in strain TA97 in the presence of metabolic activation; however, interpretation of these data is confounded by the absence of concentration-effect relationships. 4-CP was negative in assays for DNA damage in the umu test (Sakagami et al. 1988) and in a prophage induction assay with *E. coli* (DeMarini et al. 1990).

Table 2-13. Genotoxicity of 4-Chlorophenol *In Vitro*

Species (test system)	Endpoint	Results: Activation: With	Results: Activation: Without	Reference
Prokaryotic organisms:				
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Mutation	–	–	DeMarini et al. 1990
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Mutation	–	–	Haworth et al. 1983
S. typhimurium TA98, TA100	Mutation	-	-	Kubo et al. 2002
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Mutation	NA	+/–	Seuferer et al. 1979
S. typhimurium TA97, TA98, TA100, TA104	Mutation	+	+	Strobel and Grummt 1987
S. typhimurium TA1535/pSK1002	Mutation	NA	–	Rapson et al. 1980
S. typhimurium TA1535/pSK1002	Mutation	–	–	Sakagami et al. 1988
Escherichia coli WP2s(λ) (prophage induction)	DNA damage/repair	–	–	DeMarini et al. 1990
Eukaryotic organisms:				
Human primary peripheral lymphocytes (comet assay)	DNA damage	NA	–	Da Silva et al. 2007
Human primary skin fibroblasts (comet assay)	DNA damage	NA	–	Ribeiro et al. 2004
Human gingival fibroblasts (γ-H2AX assay)	DNA damage	NA	+	Shehata et al. 2012
Mouse lymphoma (L5178 cells) (comet assay)	DNA damage	NA	–	Ribeiro et al. 2004
CHO (K-1 cells) (comet assay)	DNA damage	NA	–	Ribeiro et al. 2005
SHE cells	Chromosome aberrations	+	–	Hagiwara et al. 2006

Table 2-13. Genotoxicity of 4-Chlorophenol *In Vitro*

Species (test system)	Endpoint	Results: Activation With	Results: Activation Without	Reference
SHE cells	SCE	NA	+	Miyachi and Tsutsui 2005
SHE cells	Unscheduled DNA synthesis	–	–	Hamaguchi and Tsutsui 2000
SHE cells	Morphological transformation	–	–	Yamaguchi and Tsutsui 2003

+ = positive results; +/– = equivocal results; – = negative results; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; NA = not applicable; SCE = sister chromatid exchange; SHE = Syrian hamster embryo

4-CP did not induce DNA damage in comet assays in human peripheral lymphocytes (Da Silva et al. 2007), human skin fibroblasts (Ribeiro et al. 2004), mouse lymphoma cells (Ribeiro et al. 2004), or Chinese hamster ovary (CHO) cells (Ribeiro et al. 2005). However, 4-CP induced double-stranded DNA breaks in human gingival fibroblasts as measured with the γ-H2AX focus assay (Shehata et al. 2012). The mixed results for DNA damage may stem from differences in cell type, exposure time (Shehata et al. 2012 exposed cells for 6 hours compared to 1 hour for the negative studies), or assay type (γ-H2AX versus comet assay). Both with and without metabolic activation, 4-CP failed to induce unscheduled DNA synthesis (Hamaguchi and Tsutsui 2000) or morphological transformation (Yamaguchi and Tsutsui 2003) in Syrian hamster embryo (SHE) cells. However, 4-CP induced an increase in chromosomal aberrations in SHE cells in the presence (but not in the absence) of exogenous metabolic activation (Hagiwara et al. 2006). 4-CP also induced an increased frequency of SCEs in SHE cells in the absence of exogenous metabolic activation (this assay was not conducted in the presence of metabolic activation) (Miyachi and Tsutsui 2005).

2,4-DCP. Both *in vitro* (Table 2-14) and *in vivo* assays for genotoxicity of 2,4-DCP are available. In Ames assays, 2,4-DCP was negative for mutagenic activity (Haworth et al. 1983; Kubo et al. 2002; NTP 1989; Probst et al. 1981; Rapson et al. 1980; Rasanen et al. 1977; Simmon et al. 1977; Zeiger et al. 1990), but was positive with activation in a prophage induction assay (DeMarini et al. 1990) and positive without activation in a umu test system (Ono et al. 1992). 2,4-DCP was negative for mutation in a GreenScreen assay in *Saccharomyces cerevisiae* (Knight et al. 2007). In mammalian cells, 2,4-DCP yielded negative results for mutation in Chinese hamster V79 cells (Hattula and Knuutinen 1985; Jansson and Jansson 1986) and for micronucleus induction in a human reconstructed epidermis model (EpiSkin^{TM}) (Chen et al. 2021). However, 2,4-DCP produced chromosomal aberrations in Chinese hamster V79 cells (Onfelt

1987) and CHO cells (Hilliard et al. 1998); in addition, increased chromosomal aberrations were reported in human lymphoblast (TK6) cells after exposure to cytotoxic doses of 2,4-DCP (Hilliard et al. 1998). Positive results were obtained in a test for induced unscheduled DNA synthesis in rat hepatocytes (Probst et al. 1981).

Table 2-14. Genotoxicity of 2,4-Dichlorophenol *In Vitro*

Species (test system)	Endpoint	Results: Activation With	Results: Activation Without	Reference
Prokaryotic organisms:				
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Mutation	–	–	Haworth et al. 1983; NTP 1989; Zeiger et al. 1990
S. typhimurium TA98, TA100	Mutation	–	–	Kubo et al. 2002
S. typhimurium C3076, D3052, G46, TA98, TA100, TA1535, TA1537, TA1538	Mutation	–	–	Probst et al. 1981
S. typhimurium TA100	Mutation	–	–	Rapson et al. 1980
S. typhimurium TA98, TA100, TA1535, TA1537	Mutation	–	–	Rasanen et al. 1977
S. typhimurium TA187, TA100, TA1535, TA1537, TA1538	Mutation	–	–	Simmon et al. 1977
S. typhimurium TA1535/pSK1002 (umu assay)	DNA damage/ repair	–	+	Ono et al. 1992
Escherichia coli WP2s(λ) (prophage induction assay)	DNA damage/ repair	+	–	DeMarini et al. 1990
Eukaryotic organisms:				
Chinese hamster V79 cells (with or without primary rat hepatocytes)	Mutation	–	–	Hattula and Knuutinen 1985
Chinese hamster V79 cells	Mutation	NA	–	Jansson and Jansson 1986
Human reconstructed epidermis (EpiSkin™)	Micronucleus	NA	–	Chen et Bal. 2021
Human lymphoblast (TK6)	Chromosomal aberrations	NA	+/–	Hilliard et al. 1998
Chinese hamster V79 cells	Chromosomal aberrations	NA	+	Onfelt 1987
CHO cells	Chromosomal aberrations	+	+	Hilliard et al. 1998
Rat hepatocytes	Unscheduled DNA synthesis	NA	+	Probst et al. 1981
Saccharomyces cerevisiae GenC01, GenT01 (GreenScreen assay)	DNA damage/ repair	NA	–	Knight et al. 2007

Table 2-14. Genotoxicity of 2,4-Dichlorophenol *In Vitro*

Species (test system)	Endpoint	Results: Activation: With	Results: Activation: Without	Reference

+ = positive results; +/– = equivocal results; – = negative results; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; NA = not applicable; SCE = sister chromatid exchange

Reactive intermediates produced by incubation of 2,4-DCP with horseradish peroxidase formed covalent adducts with deoxyguanosine in isolated calf thymus DNA and in isolated deoxyguanosine (Dai et al. 2005).

Galloway et al. (1998) tested whether 2,4-DCP induced chromosomal aberrations via an indirect mechanism involving inhibition of DNA synthesis. The authors used flow cytometry and BrdU uptake to assess DNA synthesis rates across the cell cycle. Following exposure to 2,4-DCP, BrdU uptake by CHO cells decreased with increasing dose, but then increased again at higher doses (Galloway et al. 1998). This result was confirmed with a repeat experiment and despite efforts by the authors to test various hypotheses (e.g., precipitation and fluorescence signaling anomalies) to explain this unexpected result, the authors were not able to account for the U-shaped dose-response (Galloway et al. 1998).

In CD-1 ICR mice, oral administration of 2,4-DCP at doses of up to 638 mg/kg/day (in corn oil by gavage for 14 days) or up to 500 mg/kg/day (in drinking water for 90 days) yielded negative results for SCE induction in testicular and bone marrow cells (respectively) (Borzelleca et al. 1985a). After five daily intraperitoneal injections of 180 mg/kg 2,4-DCP, increased percentages of chromosomal aberrations (measured 35 days after the first injection) were observed in the bone marrow and spermatocytes of Swiss mice (Amer and Aly 2001).

2,5-DCP. The genotoxicity of 2,5-DCP has been tested in both *in vitro* and *in vivo* systems. 2,5-DCP was negative for mutagenic activity in Ames assays (*S. typhimurium*) in the presence or absence of metabolic activation (Haworth et al. 1983; Kubo et al. 2002; NTP 1989; Rasanen et al. 1977) (Table 2-15). In addition, 2,5-DCP was negative for gene mutation in a GreenScreen assay in yeast (Knight et al. 2007), and negative for hypoxanthine phosphoribosyl transferase (HPRT) mutation in CHO cells both in the absence and presence of exogenous metabolic activation (Tegethoff et al. 2000). In an *in vivo* assay, male NMRI mice received a single gavage dose of 1,500 mg/kg 2,5-DCP in corn oil. Bone

marrow (femoral) micronucleus formation was assessed at 24, 48, and 72 hours post-administration, and no increase in the frequency of micronuclei was observed (Tegethoff et al. 2000).

Table 2-15. Genotoxicity of 2,5- Dichlorophenol *In Vitro*

Species (test system)	Endpoint	Results: Activation With	Results: Activation Without	Reference
Prokaryotic organisms:				
Salmonella typhimurium TA98, TA100, TA1535, TA1537	Mutation	–	–	Haworth et al. 1983; NTP 1989
S. typhimurium TA98, TA100	Mutation	–	–	Kubo et al. 2002
S. typhimurium TA98, TA100, TA1535, TA1537	Mutation	–	–	Rasanen et al. 1977
Eukaryotic organisms:				
CHO (K-1-BH4 cell line)	Mutation (HPRT locus)	–	–	Tegethoff et al. 2000

– = negative results; CHO = Chinese hamster ovary; HPRT = hypoxanthine phosphoribosyl transferase

2,4,5-TCP. Available genotoxicity data for 2,4,5-TCP include both *in vitro* and *in vivo* studies. 2,4,5-TCP was negative in most *S. typhimurium* reverse mutation assays (George et al. 1992; Kubo et al. 2002; Rasanen et al. 1977) (Table 2-16). One study (Strobel and Grummt 1987) reported increased numbers of revertants in strains TA97, TA98, and TA100, both with and without S9 fraction; however, the results lacked evidence of concentration-response relationships. Evidence of DNA damage induced by 2,4,5-TCP was reported for λ-prophage induction assays with activation (DeMarini et al. 1990), without activation (George et al. 1992), and in a umu test system both with and without activation (Ono et al. 1992). No increase in mutations was observed when 2,4,5-TCP was tested in Chinese hamster V79 cells without exogenous activation (Jansson and Jansson 1986). However, 2,4,5-TCP increased the frequency of chromosome aberrations in CHO cells both with and without metabolic activation (Armstrong et al. 1993) and induced DNA damage in human peripheral blood mononuclear cells (PBMCs) (Michalowicz and Majsterek 2010).

Table 2-16. Genotoxicity of 2,4,5-Trichlorophenol *In Vitro*

Species (test system)	Endpoint	Results: Activation With	Results: Activation Without	Reference
Prokaryotic organisms:				

Table 2-16. Genotoxicity of 2,4,5-Trichlorophenol *In Vitro*

Species (test system)	Endpoint	Results: Activation: With	Results: Activation: Without	Reference
Salmonella typhimurium TA98, TA100, TA102, TA104	Mutation	–	–	George et al. 1992
S. typhimurium TA98, TA100, TA1535, TA1537	Mutation	–	–	Haworth et al. 1983
S. typhimurium TA98, TA100	Mutation	–	–	Kubo et al. 2002
S. typhimurium TA98, TA100, TA1535, TA1537	Mutation	–	–	Rasanen et al. 1977
S. typhimurium TA98, TA100, TA97, TA104	Mutation	+	+	Strobel and Grummt 1987
S. typhimurium TA1535/psK1002 (umu assay)	DNA damage/ repair	+	+	Ono et al. 1992
Escherichia coli WP2s(λ) (prophage induction)	DNA damage/ repair	+	+	DeMarini et al. 1990; George et al. 1992
Eukaryotic organisms:				
Human PBMCs	DNA damage	NA	+	Michalowicz and Majsterek 2010
Chinese hamster V79 cells	Mutation	NA	–	Jansson and Jansson 1986
CHO cells	Chromosomal aberrations	+	+	Armstrong et al. 1993

+ = positive results; – = negative results; CHO = Chinese hamster ovary; NA = not applicable; PBMC = peripheral blood mononuclear cell

Covalent adducts with deoxyguanosine in isolated calf thymus DNA and in isolated deoxyguanosine were formed by reactive intermediates produced by incubation of 2,4,5-DCP with horseradish peroxidase (Dai et al. 2005).

In *in vivo* testing, a single gavage dose of 2,4,5-TCP (164 mg/kg) given to rats did not damage DNA as measured by the fraction of DNA eluted from white blood cells or livers (Kitchin and Brown 1988). Human peripheral blood lymphocytes from an occupational cohort of 19 herbicide production workers exposed to 2,4,5 TCP and 2,4-D showed higher frequencies (2-fold increase) of chromosomal aberrations compared with 36 control workers without chemical contact and 21 controls from the vicinity of the plant (Kaioumova and Khabutdinova 1998).

2,4,6-TCP. 2,4,6-TCP was tested for genotoxicity in both *in vitro* and *in vivo* assays. 2,4,6-TCP did not induce mutations in *S. typhimurium* (Ames) assays in the presence or absence of metabolic activation in the preponderance of available studies (Haworth et al. 1983; Kinae et al. 1981; Kubo et al. 2002; Rapson

et al. 1980; Rasanen et al. 1977) (Table 2-17). Strobel and Grummt (1987) reported increased mutations in *S. typhimurium* TA97, TA98, and TA104 with exogenous metabolic activation; however, the results lacked evidence of concentration-response relationships. In umu assay testing, 2,4,6-TCP did not induce DNA damage with activation but did induce damage without activation (Ono et al. 1992). Positive results were also reported for DNA damage in a prophage induction assay both with and without activation (DeMarini et al. 1990) and in a bacterial (*Bacillus subtilis*) assay of DNA damage (Kinae et al. 1981).

Table 2-17. Genotoxicity of 2,4,6-Trichlorophenol *In Vitro*

		Results		
		Activation		
Species (test system)	Endpoint	With	Without	Reference
Prokaryotic organisms:				
Salmonella typhimurium TA100, TA1535, TA1537	Mutation	–	–	Haworth et al. 1983
S. typhimurium TA98, TA100, TA1537	Mutation	–	–	Kinae et al. 1981
S. typhimurium TA98, TA100	Mutation	–	–	Kubo et al. 2002
S. typhimurium TA100	Mutation	NA	–	Rapson et al. 1980
S. typhimurium TA98, TA100, TA97, TA104	Mutation	+	–	Strobel and Grummt 1987
S. typhimurium TA98, TA100, TA1535, TA1537	Mutation	–	–	Rasanen et al. 1977
S. typhimurium TA1535/pSK1002 (umu test)	DNA damage/repair	–	+	Ono et al. 1992
Escherichia coli WP2(λ) (prophage induction)	DNA damage/repair	+	+	DeMarini et al. 1990
Bacillus subtilis H-17, M-45	DNA damage	NA	+	Kinae et al. 1981
Eukaryotic organisms:				
Mouse (L5178Y TK+/- cells)	Mutation	NA	+	McGregor et al. 1988
Chinese hamster V79 cells	Mutation	NA	–	Jansson and Jansson 1992, 1986
Chinese hamster V79 cells (with or without primary rat hepatocytes)	Mutation	–	+	Hattula and Knuutinen 1985
Saccharomyces cerevisiae MP-1	Mutation	–	+	Fahrig et al. 1978
CHO cells	Chromosomal aberrations	+	+	Armstrong et al. 1993
CHO cells	SCEs and chromosomal aberrations	NA	–	Galloway et al. 1987
Chinese hamster V79 cells	Chromosomal aberrations	NA	+	Jansson and Jansson 1992
Chinese hamster CHL/IU cells	Chromosomal aberrations	+	–	Matsuoka et al. 1998

Table 2-17. Genotoxicity of 2,4,6-Trichlorophenol *In Vitro*

Species (test system)	Endpoint	Results: Activation: With	Results: Activation: Without	Reference
Mouse embryonic fibroblasts (C3H10T1/2)	DNA damage	+/–	–	Wang and Lin 1995
S. cerevisiae MP-1	Mitotic crossing over or gene conversion	NA	–	Fahrig et al. 1978

+ = positive results; +/– = borderline mutagen; – = negative results; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; NA = not applicable; SCE = sister chromatid exchange

In testing in yeast cells (*S. cerevisiae*), 2,4,6-TCP induced mutations in the absence (but not in the presence) of activation but showed no evidence for increased mitotic gene conversion or mitotic crossing over (Fahrig et al. 1978). *In vitro* evaluations of mutation yielded positive results in the absence of metabolic activation in Chinese hamster V-79 cells (Hattula and Knuutinen 1985) and mouse lymphoma L5178Y TK +/- cells (McGregor et al. 1988), and negative results in the presence of metabolic activation in Chinese hamster V-79 cells (Hattula and Knuutinen 1985; Jansson and Jansson 1992).

2,4,6-TCP treatment resulted in increased frequencies of chromosomal aberrations in Chinese hamster V79 cells without metabolic activation (Jansson and Jansson 1992). Mixed results were obtained with CHO cells; Armstrong et al. (1993) reported increased chromosomal aberrations both with and without metabolic activation, while Galloway et al. (1987) reported negative results in assays for both chromosomal aberrations and SCEs in the absence of metabolic activation. No increase in chromosomal aberrations was observed in Chinese hamster CHL/IU cells exposed to 2,4,6-TCP in the absence of activation, but positive results were seen with activation (Matsuoka et al. 1998). Equivocal or negative results were reported in testing for DNA damage in mouse embryonic fibroblasts (Wang and Lin 1995).

As was seen with 2,4-DCP and 2,4,5-TCP, incubation of 2,4,6-TCP with horseradish peroxidase resulted in the formation of reactive intermediates that formed covalent adducts with deoxyguanosine in isolated calf thymus DNA and in isolated deoxyguanosine (Dai et al. 2005).

In vivo, 2,4,6-TCP demonstrated genotoxic activity in somatic cells of mice in the spot test (Fahrig et al. 1978). A single gavage dose of 2,4,6-TCP (164 mg/kg) to rats did not damage DNA as measured by alkaline elution of DNA from white blood cells or livers (Kitchin and Brown 1988). *In vivo* tests of 2,4,6-TCP using insect systems (*Drosophila melanogaster*) were also negative (Valencia et al. 1985).

2,3,4,6-TeCP. Both *in vitro* and *in vivo* genotoxicity data are available for 2,3,4,6-TeCP. 2,3,4,6 TeCP tested negative for mutation in Ames assays (Rasanen et al. 1977; Zeiger et al. 1988) and tested negative in a prophage induction assay (DeMarini et al. 1990). However, 2,3,4,6-TeCP was positive both with and without activation in a umu test system (Ono et al. 1992). 2,3,4,6-TeCP did test positive for mutation in Chinese hamster V79 cells (Hattula and Knuutinen 1985). When tested with hydrogen peroxide, 2,3,4,6-TeCP induced DNA damage in human fibroblast GM5757 cells (Lueken et al. 2004). A single gavage dose of 2,3,4,6-TeCP (28 or 193 mg/kg) given to rats did not damage DNA in white blood cells or livers as measured by the alkaline elution assay (Kitchin and Brown 1988).

Other Chlorophenols. In tests using the umu assay, 2,3-DCP was negative both with and without S9 fraction, while both 3,4- and 3,5-TCP were negative with activation and positive without activation (Ono et al. 1992) (Table 2-18). In the same study, positive results both with and without metabolic activation were reported for 2,3,4-TCP (Ono et al. 1992). 2,3,4-TCP did not induce reverse mutations in Ames assays (Zeiger et al. 1992). In Chinese hamster lung cells treated with 2,3,4-TCP, there was no treatment-related increase in chromosomal aberrations in the presence or absence of metabolic activation; however, chromosomal aberrations were increased in CHO cells by treatment with 2,3,4-TCP in the presence of metabolic activation (Sofuni et al. 1990).

Table 2-18. Genotoxicity of Other Chlorophenols *In Vitro*

Species (test system)	Endpoint	Results: Activation With	Results: Activation Without	Reference	Compound (purity)
Prokaryotic organisms:					
Salmonella typhimurium TA1535/psK1002 (umu test)	DNA damage/ repair	–	–	Ono et al. 1992	2,3-DCP
S. typhimurium TA1535/psK1002 (umu test)	DNA damage/ repair	–	+	Ono et al. 1992	3,4-DCP
S. typhimurium TA1535/psK1002 (umu test)	DNA damage/ repair	–	+	Ono et al. 1992	3,5-DCP
S. typhimurium TA98, TA100, TA1535, and TA97 or TA1537	Mutation	–	–	Zeiger et al. 1992	2,3,4-TCP
S. typhimurium TA1535/psK1002 (umu test)	DNA damage/ repair	+	+	Ono et al. 1992	2,3,4-TCP
S. typhimurium TA97, TA98, TA100, TA1535	Mutation	–	–	Zeiger et al. 1988	2,3,4,5-TeCP
Escherichia coli WP2s(λ) (prophage induction)	DNA damage/ repair	+	–	DeMarini et al. 1990	2,3,4,5-TeCP

Table 2-18. Genotoxicity of Other Chlorophenols *In Vitro*

Species (test system)	Endpoint	Results		Reference	Compound (purity)
		Activation			
		With	Without		
S. typhimurium Ta97, TA98, TA100, TA1535	Mutation	–	–	Zeiger et al. 1988	2,3,5,6-TeCP
E. coli WP2s(λ) (prophage induction)	DNA damage/ repair	–	–	DeMarini et al. 1990	2,3,5,6-TeCP
Eukaryotic organisms:					
Chinese hamster lung cells	Chromosomal aberrations	–	–	Sofuni et al. 1990	2,3,4-TCP
CHO cells	Chromosomal aberrations	+	–	Sofuni et al. 1990	2,3,4-TCP
Chinese hamster lung cells	Chromosomal aberrations	+	–	Sofuni et al. 1990	2,3,4,5-TeCP
CHO cells	Chromosomal aberrations	–	–	Sofuni et al. 1990	2,3,4,5-TeCP
Chinese hamster lung cells	Chromosomal aberrations	+	–	Sofuni et al. 1990	2,3,5,6-TeCP
CHO cells	Chromosomal aberrations	+	–	Sofuni et al. 1990	2,3,5,6-TeCP

+ = positive results;– = negative results; CHO = Chinese hamster ovary; CP = chlorophenol; DCP = dichlorophenol; DNA = deoxyribonucleic acid; TCP = trichlorophenol; TeCP = tetrachlorophenol

Both 2,3,4,5- and 2,3,5,6-TeCP were negative for mutation in *S. typhimurium* with and without activation (Zeiger et al. 1988). In λ-prophage induction assays, results for 2,3,4,5- and 2,3,5,6-TeCP were negative in the absence and presence of metabolic activation (DeMarini et al. 1990). 2,3,4,5-TeCP induced an increase in chromosomal aberrations in Chinese hamster lung cells with (but not without) exogenous metabolic activation, but not in CHO cells (Sofuni et al. 1990). In contrast, 2,4,5,6-TeCP increased chromosomal aberrations in both cell types when tested with metabolic activation (Sofuni et al. 1990).