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 CDFs. 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.

As noted in Chapter 1, this toxicological profile focuses on the tetra-, penta-, hexa-, hepta-, and octa-chlorinated CDF congeners. Although humans are exposed to mixtures of numerous CDF congeners, animal studies involving exposure to a single CDF congener are only available for eight congeners: 2,3,7,8-tetraCDF, 1,2,3,4,8-pentaCDF, 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, 1,2,3,4,7,8-hexaCDF, 1,2,3,6,7,8-hexaCDF, 1,2,3,4,6,7,8-heptaCDF, and octaCDF.

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

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

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to CDFs, but may not be inclusive of the entire body of literature.

Animal oral studies are presented in Table 2-2 and Figure 2-2. Table 2-1 is divided by exposure duration and by congener. Animal dermal studies are presented in Table 2-3; no inhalation data were identified for CDFs.

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 were 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 CDFs are indicated in Tables 2-2 and 2-3 and Figure 2-2.

A User's Guide is 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.

Much of the information that pertains to human health effects of CDFs comes from large numbers of people who consumed rice oil contaminated with PCB-containing heat exchange fluid in Japan in 1968 (Yusho incident) and Taiwan in 1979 (Yu-Cheng incident) (Chen and Hsu 1986; Kuratsune 1989; Kashimoto and Miyata 1986; Okumura 1984; Rogan 1989). The PCB-containing fluid was heated in thermal heat exchangers before contamination occurred and also during cooking resulting in the production of relatively high concentrations of CDFs and polychlorinated quaterphenyl (PCQ) impurities by thermal degradation. Yusho involved at least 1,854 victims exposed over ≈10 months, and Yu-Cheng involved at least 2,061 victims exposed over ≈9 months (Chen et al. 1985a; Hsu et al. 1984; Kuratsune 1989; Rogan 1989). The concentrations of PCBs and PCQs in the rice oils were l00- to 500-fold greater than the CDFs. Because there are no data on human health effects of CDFs alone and little is known about any potential interactive effects between CDFs and other components of the contaminated rice oil mixtures, the health effects in Yusho and Yu-Cheng victims cannot be attributed solely to CDFs.

However, CDFs are generally considered to be the main causal agent based predominantly on: (1) comparisons with Japanese workers with higher PCB blood levels who had few or none of the symptoms present in the rice oil poisonings, (2) decreasing serum levels of PCBs in victims with persisting health effects, (3) induction of Yusho health effects in animals exposed to reconstituted mixtures of CDF congeners similar to those in Yusho oils, but not by exposure to PCBs or PCQs alone, and (4) comparative toxicity evaluations of PCB and CDF congeners in unheated source mixtures, contaminated rice oil, and tissues of victims (Bandiera et al. 1984a; Kunita et al. 1984; Masuda and Yoshimura 1984; Ryan et al. 1990; Safe 1990a; Takayama et al. 1991; Tanabe et al. 1989).

In general, clinical severity of signs and symptoms was closely related to the total amount of oil consumed, but not to the amount consumed per kg body weight per day (Hayabuchi et al. 1979; Kuratsune 1989). Concentrations of CDFs in the Yu-Cheng oil were much lower than in the Yusho oil, and intake of Yu-Cheng oil was believed to be much higher than for Yusho oil (Chen et al. 1985a). This resulted in very similar estimated average total intakes of PCBs, CDFs, and PCQs of 633, 3.3, and 596 mg, respectively, for Yusho (Hayabuchi et al. 1979), and 973, 3.8, and 586 mg, respectively, for Yu-Cheng (Chen et al. 1985a). Based on the Yusho intake, the average daily amount of CDFs ingested per kg body weight was 0.9 µg/kg/day (Hayabuchi et al. 1979). Of more than 40 CDF congeners present in Yusho and Yu-Cheng oils, the two major congeners that accumulated in the victims were 2,3,4,7,8-pentaCDF and 1,2,3,4,7,8-hexaCDF. Contributions of other 2,3,7,8-chlorine substituted CDF congeners to the toxic effects are not considered to be substantial since they were not present in significant amounts in the rice oils, were not detectably accumulated in human tissues, and/or were of lower potency (Ryan et al. 1990).

The general population is not typically exposed to single CDF congeners, rather they are environmentally exposed to mixtures of halogenated aromatic hydrocarbons, of which various CDFs are constituents. CDDs and PCBs frequently occur with CDFs in the environment. The toxic effects of CDDs, CDFs, and some non-*ortho*-substituted PCBs (collectively referred to as dioxin-like compounds or dioxins) share a common mechanism of action in that they are mediated through the Ah receptor, resulting in similar adverse health outcomes. Although they share toxic endpoints, there are congener-specific differences in toxic potency. Experimental data evaluating the toxicity of mixtures of dioxin-like compounds provide strong evidence of additivity (van den Berg et al. 2006). To provide an estimate of the toxic potency of mixtures of these compounds while accounting for the toxic potency differences between them, a TEF approach was developed.

In the TEF approach for dioxin-like compounds, the relative effect potency of individual CDD, CDF, and PCB congeners for producing toxic or biological effects is estimated and expressed relative to that of the reference compound, 2,3,7,8-TCDD (TEF=1). The TEFs can be used, assuming additivity of the toxic response, for estimating the toxicity of an environmental mixture containing a known distribution of CDFs, CDDs, and/or PCBs. Given the assumption of additivity of the toxic responses, the total toxic equivalent (TEQ) of a mixture is defined as the sum of the products of the concentration of each mixture component multiplied by its respective TEF. The resulting TEQ value is an estimate of the total 2,3,7,8-TCDD-like activity of the mixture (van den Berg et al. 2006).

An expert panel organized by the World Health Organization (WHO) initially developed TEFs for all 2,3,7,8-substituted CDDs and CDFs and several PCBs in 1993, and subsequent WHO expert panels updated these TEFs in 1998 and 2005. In the 2005 TEFs, PCB compounds were included if they met the following criteria: (1) they show a structural relationship to CDDs and CDFs; (2) they bind to the Ah receptor; (3) they elicit Ah receptor-mediated biochemical and toxic responses; and (4) they are persistent and accumulate in the food chain (van den Berg et al. 2006). For additional information on the development of the TEFs, see Haws et al. (2006) and van den Berg et al. (2006). The 1998 and 2005 WHO TEFs are presented in Table 2-1.

Table 2-1. Summary of World Health Organization (WHO) 1998 and 2005 Toxicity Equivalency Factors (TEFs)

Compound	1998 TEF[a]	2005 TEF[a]
Chlorinated dibenzo-*p*-dioxins (CDDs)		
2,3,7,8-TCDD	1	1
1,2,3,7,8-PentaCDD	1	1
1,2,3,4,7,8-HexaCDD	0.1	0.1
1,2,3,6,7,8-HexaCDD	0.1	0.1
1,2,3,7,8,9-HexaCDD	0.1	0.1
1,2,3,4,6,7,8-HeptaCDD	0.01	0.01
OctaCDD	0.0001	0.0003
Chlorodibenzofurans (CDFs)		
2,3,7,8-TetraCDF	0.1	0.1
1,2,3,7,8-PentaCDF	0.05	0.03
2,3,4,7,8-PentaCDF	0.5	0.3
1,2,3,4,7,8-HexaCDF	0.1	0.1
1,2,3,6,7,8-HexaCDF	0.1	0.1
1,2,3,7,8,9-HexaCDF	0.1	0.1
2,3,4,6,7,8-HexaCDF	0.1	0.1

Table 2-1. Summary of World Health Organization (WHO) 1998 and 2005 Toxicity Equivalency Factors (TEFs)

Compound	1998 TEF[a]	2005 TEF[a]
1,2,3,4,6,7,8-HeptaCDF	0.01	0.01
1,2,3,4,7,8,9-HeptaCDF	0.01	0.01
OctaCDF	0.0001	0.0003
Non-*ortho*-substituted polychlorinated biphenyls (PCBs)		
3,3′,4,4′-TetraCB (PCB 77)	0.0001	0.0001
2,3,4,4′,5-TetraCB (PCB 81)	0.0001	0.0003
3,3′,4,4′,5-PentaCB (PCB 126)	0.1	0.1
3,3′,4,4′,5,5′-HexaCB (PCB 169)	0.01	0.03
Mono-*ortho*-substituted polychlorinated biphenyls (PCBs)		
2,3,3′,4,4′-pentaCB (PCB105)	0.0001	0.00003
2,3, 4,4′,5-pentaCB (PCB114)	0.0005	0.00003
2,3′,4,4′,5-pentaCB (PCB118)	0.0001	0.00003
2′,3,4,4′,5-pentaCB (PCB123)	0.0001	0.00003
2,3,3′,4,4′,5-hexaCB (PCB156)	0.0005	0.00003
2,3,3′,4,4′,5′-hexaCB (PCB157)	0.0005	0.00003
2,3′,4,4′,5,5′-hexaCB (PCB167)	0.000001	0.00003
2,3,3′,4,4′,5,5′-heptaCB (PCB189)	0.0001	0.00003

[a]TEFs are relative to the toxicity of 2,3,7,8-TCDD.

Source: van den Berg et al. 1998, 2006

The epidemiological database evaluating the toxicity of CDDs, CDFs, and/or PCBs is extensive. The database consists of occupational exposure studies, studies of communities living near point sources, communities affected by accidental releases, and the general population exposed to background levels, primarily from CDDs, CDFs, and/or PCBs in the food supply. These studies have identified a number of adverse health outcomes associated with exposure to dioxin-like compounds. The health outcomes include chloracne; evidence of liver damage including increases in serum hepatic enzyme levels, hepatomegaly, and increases in serum lipid levels; evidence of altered thyroid function; increased risk of diabetes and abnormal glucose tolerance tests; alterations in immune endpoints; endometriosis; altered offspring sex ratio; and neurodevelopmental alterations, including ototoxicity (Akahane et al. 2018; ATSDR 1998, 2000, 2012). The results of these studies should be interpreted cautiously particularly because some of the outcomes were still within the range found in unexposed populations. With the exception of some occupational exposure and community exposure studies, exposure levels were not measured; most studies used serum CDF, CDD, and/or PCB levels as a biomarker for exposure. Studies reported serum levels as individual congener levels; total CDD, CDF, and/or PCB levels; total CDD/CDF

levels; TEFs for individual congeners; and total CDD/CDF or CDD/CDF/PCB TEQs. The discussion of epidemiological data in this profile is limited to studies of known exposure (e.g., Yusho and Yu-Cheng incidents) and studies measuring serum levels of CDF congeners, total CDF congeners, CDF congener TEQs, or total CDF TEQ.

Because humans are exposed to a mixture of dioxin-like compounds, it is difficult to ascribe an effect to a particular compound or congener; rather it is likely that many, if not all, contribute to the adverse health outcomes. None of the available CDF epidemiological studies controlled for co-exposure to other dioxin-like compounds or other chemicals. Some of the studies, but not all, controlled for confounders that may affect the outcome such as age, smoking history, etc. The epidemiological data are not adequate to establish causality. Although some studies found associations or inverse associations between serum CDF levels and health outcomes, the studies were often limited by their design (cross sectional), the small number of individuals examined, inconsistent results, or there were too few studies to evaluate the weight of evidence.

Information regarding health effects in animals exposed to CDFs was located for the following congeners: 2,3,7,8-tetraCDF, 1,2,3,4,8-pentaCDF, 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, 1,2,3,4,7,8-hexaCDF, 1,2,3,6,7,8-hexaCDF, 1,2,3,4,6,7,8-heptaCDF, and 1,2,3,4,6,7,8,9-octaCDF.

As illustrated in Figure 2-1, there are epidemiological and laboratory animal data available for the 18 health endpoints discussed in the toxicological profile. The most studied endpoints are developmental, immunological, and hepatic. Most of the epidemiological studies did not provide information on the route of exposure; for environmental exposures, it was assumed to be oral exposure. Over 95% of the epidemiological and toxicological studies involved oral exposure to CDFs. As noted earlier, humans are exposed to a mixture of CDF congeners. Most laboratory animal studies involved exposure to a single congener, although there are several studies examining effects associated with exposure to a mixture of CDF congeners. Of the over 50 laboratory animal toxicity studies, 45% evaluated the toxicity of 2,3,4,7,8-pentaCDF and 26% evaluated 2,3,7,8-tetraCDF. Four congeners (1,2,3,4,8-pentaCDF, 1,2,3,4,7,8-hexaCDF, 1,2,3,4,6,7,8-heptaCDF, and octaCDF) each had only one study.

The human and animal studies suggest several sensitive targets of CDFs toxicity:

- **Hepatic Endpoints.** Hepatic effects are observed in humans and animals orally exposed to CDFs. The effects include increases in liver weight, lipid accumulation and hypertrophy in the liver, and alterations in serum triglyceride levels.

- **Immunological Endpoints.** Clinical observations of increased susceptibility to respiratory and dermal infections and various changes in immune parameters, including decreased antibody and leukocyte levels and delayed-type skin hypersensitivity response, have been observed in Yusho and Yu-Cheng cohorts. Studies in animals indicate that the immunological system may be the most sensitive to effects caused by CDFs. Pronounced decreases in thymus weight and/or histologic thymic atrophy were consistently observed following oral exposure in all tested species. There are also limited data suggesting that CDFs impair immune responses to mitogens.

- **Thyroid Endpoints.** A small number of epidemiological studies examined potential thyroid effects; the small number of studies limits drawing conclusions. Decreases in serum T4 levels were reported in rats exposed to tetra- and pentaCDF congeners.

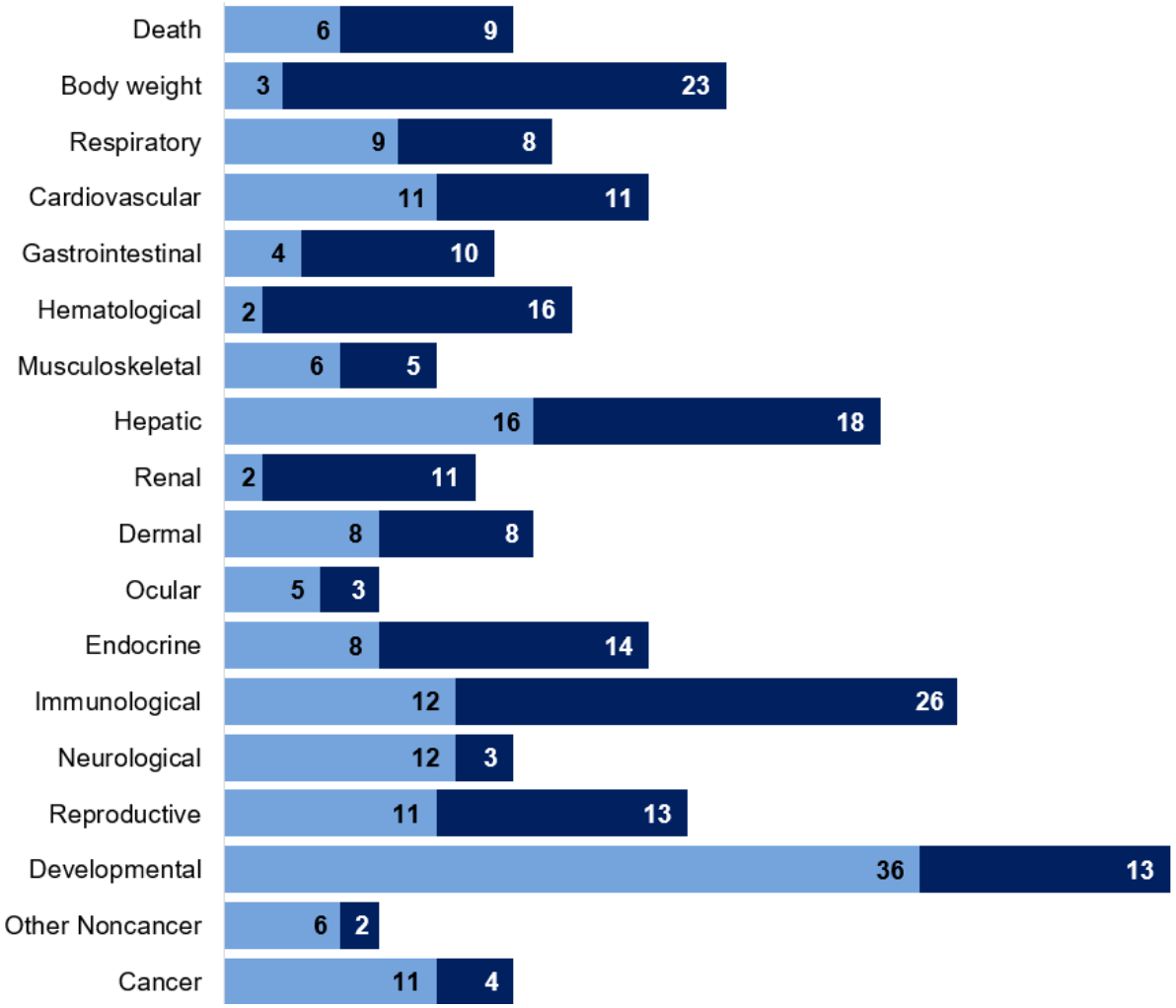
- **Developmental Endpoints.** Various signs of toxicity have been observed in children born to mothers exposed during the Yusho and Yu-Cheng incidents. Toxic effects include dermal lesions similar to those found in exposed adults, perinatal deaths in some babies with dermal lesions, decreased birth weights, and neurobehavioral deficits. Developmental effects observed in animals include hydronephrosis and cleft palate in mice, fetal mortality, decreases in fetal weights, and impaired development of the reproductive system.

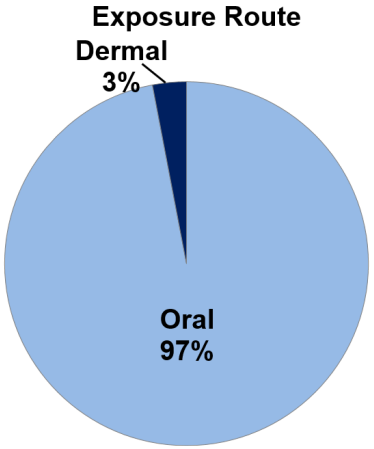
The discussions of the available data for each health effect in Sections 2.2 through 2.19 are divided into several subsections. Each health effect section begins with a discussion of epidemiological data, if available. Congener-specific discussions of laboratory animal data follow. It is noted that for most health effects, there are no data for a number of congeners.

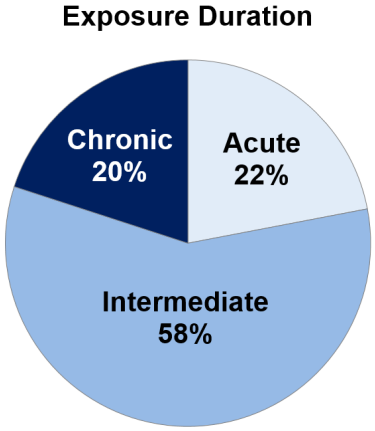
Figure 2-1. Overview of the Number of Studies Examining Chlorodibenzofurans (CDFs) Health Effects*

Most studies examined the potential immunological, hepatic, and developmental effects of CDFs

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







*Includes studies discussed in Chapter 2. A total of 147 studies (including those finding no effect) examined toxicity; most studies examined multiple endpoints and a number of animal studies examined several congeners.

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
2,3,7,8-TetraCDF									
1	Monkey (Rhesus) 2–4 F	1 day (GO)	0, 500, 1,000, 1,500	BW GN HP BC CS BI	Death			1,000	2/4 animals died at 1,000 µg/kg and 2/2 died at 1,500 µg/kg
					Bd wt		500	1,000	LOAEL: decreased weight gain (magnitude not reported) Serious LOAEL: weight loss in surviving monkeys at 1,000 µg/kg
					Resp	1,500			
					Cardio	1,500			
					Gastro	500	1,000		Loss of parietal cells, increase in mucous cells, and microcystic dilation of crypts in the glandular stomach
					Hemato		500		Mild anemia, lymphopenia, neutrophilia
					Hepatic	500	1,000		Gall bladder and bile duct hypertrophy
					Renal	1,500			
					Dermal		500		Facial edema, occluded or dilated ceruminous and sebaceous glands, nail loss, epidermal hyperkeratosis
					Ocular		500		Occluded or dilated meibomian glands, eyelash loss
					Endocr	1,500			
					Immuno	500	1,000		Thymus and spleen atrophy
					Neuro	1,500			

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Other noncancer		1,000		Degranulation of exocrine pancreatic cells in animals dying early
2,3,7,8-TetraCDF Moore et al. 1979									
2	Rat (Long-Evans) 4–14 F	4 days (GO)	0.3–100	BW BC	Endocr		4.65		30% decrease in serum total T4 levels
2,3,7,8-TetraCDF Crofton et al. 2005									
3	Rat (Long-Evans) NS F	4 days (G)	0.3, 1, 3, 10, 30, 100	BC	Endocr	0.3	1		Decreased serum total T4 levels (approximately 26, 17, 50, 53, and 55% at 1, 3, 10, 30, and 100 µg/kg/day, respectively)
2,3,7,8-TetraCDF Ross et al. 2000									
4	Mouse (C57BL/6fh) 8 M	1 day (GO)	0, 400, 600, 800, 1,200, 1,500, 2,500, 4,000, 6,000	HP CS	Resp	6,000			
					Cardio	6,000			
					Gastro	6,000			
					Musc/skel	6,000			
					Renal	6,000			
					Dermal	6,000			
					Endocr	6,000			
2,3,7,8-TetraCDF Moore et al. 1976, 1979									
5	Mouse (C57BL/6N) 6 F	GD 10 (GO)	0, 250, 500, 1,000	BW OW CS FX MX DX	Develop			250	Fetal mortality, hydronephrosis
2,3,7,8-TetraCDF Weber et al. 1984									

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
6	Mouse (C57BL/6N) 7–11 F	GD 10 (GO)	0, 300, 600, 900	BW OW CS FX MX DX	Develop			300	Hydronephrosis; cleft palate at ≥600 µg/kg
2,3,7,8-TetraCDF Weber et al. 1985									
7	MOUSE (C57BL/6N) 8–11 F	GDs 10–13 (GO)	0, 10, 30, 50, 100	BW OW CS FX MX DX	Develop			10	Hydronephrosis; cleft palate at ≥50 µg/kg
2,3,7,8-TetraCDF Weber et al. 1984									
8	Guinea pig (Hartley) 6 M	1 day (GO)	0, 1, 5, 10, 15	BW GN HP BC CS	Death			10	100% mortality; mean time to death was 14.6 days
					Bd wt		1	10	LOAEL: decreased body weight gain (magnitude not reported) Serious LOAEL: rapid and progressive weight loss
					Resp	15			
					Cardio	15			
					Gastro	15			
					Hemato	15			
					Musc/skel		5		Reduction in muscle mass
					Renal	5	10		Hyperplasia of epithelial cells in renal pelvis, ureter, and urinary bladder
					Dermal	15			
					Ocular	15			
					Endocr	5	10		Adrenal hemorrhage

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Immuno		5		Marked reduction in size of thymus at ≥5 µg/kg; loss of lymphoid cells in thymic cortex and hypocellularity of bone marrow and lymphoid elements in spleen and Peyers patches at ≥10 µg/kg
					Neuro	15			
					Repro	5	10		Hypocellularity of seminiferous tubules
2,3,7,8-TetraCDF **Moore et al. 1979**									
1,2,3,7,8-PentaCDF									
9	Rat (Long-Evans) 4–14 F	4 days (GO)	0.03–100	BW BI	Endocr		15.6		30% decrease in serum total T4 levels
1,2,3,7,8-PentaCDF **Crofton et al. 2005**									
10	Rat (Long-Evans) NS F	4 days (G)	0.3, 1, 3, 10, 30, 100	BC	Endocr	3	10		Decreased serum total T4 levels (approximately 15, 40, and 33% at 10, 30, and 100 µg/kg/day, respectively)
1,2,3,7,8-PentaCDF **Ross et al. 2000**									
11	Mouse (C57BL/6N) 10–13 F	GDs 10–13 (GO)	0, 1, 3, 10, 30, 100, 150, 200	BW OW CS FX MX DX	Bd wt	30	100		Decreased maternal weight gain (30%)
					Develop	10		30	Hydronephrosis; cleft palate at ≥100 µg/kg/day
1,2,3,7,8-PentaCDF **Birnbaum et al. 1987a**									

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
2,3,4,7,8-PentaCDF									
12	Rat (Wistar) 8–10 F	GD 16 (GO)	0, 0.5, 2, 10	OW BI FX	Develop	0.5[b]	2		14% decreased relative neonatal thymus weight
2,3,4,7,8-PentaCDF Madsen and Larsen 1989									
13	Rat (Sprague-Dawley) 5 M	1 day (GO)	0, 53	BW OW BI	Bd wt	53			
					Hepatic	53			
					Immuno	53			
2,3,4,7,8-PentaCDF Ahlborg et al. 1989									
14	Rat (Fischer-344) 8 M	1 day (GO)	0, 100, 250, 500, 1,000, 2,000	BW OW HP BC CS BI UR	Death			916	
					Bd wt	250	500		17% lower terminal body weight
					Resp	2000			
					Gastro	250	500		Epithelial hyperplasia of nonglandular stomach
					Hemato		100		Decreased hemoglobin (6%), MCH (9%), and MCV (4%) 35 days post-exposure
					Hepatic		100		Lipid accumulation, increased serum cholesterol (60%) 35 days post-exposure
					Renal	1,000	2,000		Increased BUN (64%), increased relative kidney weight (34%)
					Dermal		500		Nail hemorrhages

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Immuno		100	500	LOAEL: decreased thymus weight (30–90%) Serious LOAEL: thymic atrophy and lymphoid depletion in the thymus and spleen
					Repro	2,000			
2,3,4,7,8-PentaCDF **Brewster et al. 1988**									
15	Rat (F344) 9–12 F	GDs 8,10, or 12 (GO)	0, 10, 30, 100, 300	BW OW CS FX MX DX	Develop		30	100	LOAEL: decreased fetal weight Serious LOAEL: increased fetal mortality
2,3,4,7,8-PentaCDF **Couture et al. 1989**									
16	Rat (Long-Evans) 4–14	4 days (GO)	0.03–90	BW BC	Endocr		27.5		30% decrease in serum total T4 levels
2,3,4,7,8-PentaCDF **Crofton et al. 2005**									
17	Rat (Long-Evans) NS F	4 days (G)	0.03, 0.09, 0.3, 0.9, 3, 9, 30, 90	BC	Endocr	9	30		Decreased serum total T4 levels (approximately 26 and 47% at 30 and 90 µg/kg/day, respectively)
2,3,4,7,8-PentaCDF **Ross et al. 2000**									
18	Rat (Sprague-Dawley) 3–4 F	GD 15 (GO)	0, 1.0, 10.0	BW RX	Develop			1	Decreased offspring body weight on PND 140, decreased number of days spent in estrus, and decreases in ovulation rate
2,3,4,7,8-PentaCDF **Salisbury and Marcinkiewicz 2002**									

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
19	Rat (Wistar) 3–13 F	GD 15 (GO)	0, 1, 2, 5, 10, 15, 25, 50, 300, 1,000	BC BW DX	Develop		12.6		ED_{50} for reduction in growth hormone levels in female fetuses; ED_{50} in male fetuses was 27.4 µg/kg; ED_{50} values for serum LH levels were 21.5 and 25.5 µg/kg in male and female fetuses, respectively; and ED_{50} values for decreases in fetal body weights were 56.3 and 140 µg/kg in male and females, respectively
2,3,4,7,8-PentaCDF Taura et al. 2014									
20	Rat (Wistar) 3–13 F	GD 15 (GO)	0, 15, 50	DX	Develop	15	50		Altered sexual behavior in male offspring (increases in mount latency and latency until first intromission and decreases in mount frequency and intromission frequency)
2,3,4,7,8-PentaCDF Taura et al. 2014									
21	Rat (Wistar) NS M	1 day (GO)	0, 1, 5, 50, 150, 500, 1,000, 2,000	BW OW	Bd wt		146		ED_{50} for decrease in body weight gain in pubertal rats
					Immuno		71.9		ED_{50} for decrease in thymus weight in pubertal rats
2,3,4,7,8-PentaCDF Taura et al. 2014									
22	Mouse (C57BL/ 6N) NS F	GDs 10–13 (GO)	0, 5, 10, 30	BW OW CS FX MX DX	Develop			5	Hydronephrosis; cleft palate at 30 µg/kg/day
2,3,4,7,8-PentaCDF Birnbaum et al. 1987b									

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
23	Mouse (C57BL/6N) 11–20 F	GDs 10–13 (GO)	0, 1, 3, 10, 20, 30, 40, 60, 80	BW OW CS MX DX	Develop	3		10	Hydronephrosis; cleft palate at ≥30 µg/kg/day
2,3,4,7,8-PentaCDF Birnbaum et al. 1987a									
24	Mouse (C57BL/6N) 2 F	GDs 10–13 (GO)	0, 80	HP MX	Repro		80		Rupture of the placental labyrinth barrier and transplacental passage of embryonic erythrocytes into maternal blood
					Develop		80		Impaired embryonic erythropoiesis in the liver, increased number of hepatocytes, and reduction in liver sinusoids
2,3,4,7,8-PentaCDF Khera 1992									
25	Mouse (B6C3F1) 14 F	1 day (GO)	0, 3, 9, 15, 30, 90	BW OW IX	Bd wt	90			
					Immuno		10.119		50% reduction in immune response to SRBC
2,3,4,7,8-PentaCDF Johnson et al. 2000									
26	Guinea pig (Hartley) 6 M	1 day (GO)	0, 1, 3, 10, 30	BW GN HP CS	Death			10	
					Bd wt		1	10	LOAEL: decreased body weight gain (magnitude not reported) Serious LOAEL: rapid and progressive weight loss
					Resp	30			
					Cardio	30			
					Gastro	30			
					Hemato	30			

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Musc/skel		3		Reduction in muscle mass
					Renal	3	10		Hyperplasia of epithelial cells in renal pelvis, ureter, and urinary bladder
					Dermal	30			
					Ocular	30			
					Endocr	3	10		Adrenal hemorrhage
					Immuno		3		Marked reduction in size of thymus at ≥3 µg/kg; at ≥10 µg/kg, loss of lymphoid cells in thymic cortex and hypocellularity of bone marrow and lymphoid elements in spleen and Peyers patches
					Neuro	30			
					Repro	3	10		Hypocellularity of seminiferous tubules
2,3,4,7,8-PentaCDF Moore et al. 1979									
1,2,3,4,7,8-HexaCDF									
27	Mouse (C57BL/6N) 10–13 F	GDs 10–13 (GO)	0, 100, 200, 300, 600, 1,000	BW OW CS FX MX DX	Develop			100	Hydronephrosis and increased fetal weight; cleft palate at ≥300 µg/kg/day
1,2,3,4,7,8-HexaCDF Birnbaum et al. 1987a									
28	Mouse (C57BL/6N) 9–13 F	GDs 10–13 (GO)	0, 100, 200, 300	BW OW CS FX MX DX	Develop			100	Hydronephrosis
1,2,3,4,7,8-HexaCDF Birnbaum et al. 1987b									

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
1,2,3,4,6,7,8-HeptaCDF									
29	Mouse (C57BL/6N) 6 NS	1 day (GO)	0, 25, 100, 600	IX	Immuno		208		ED_{50} for decreased antibody response to SRBC
1,2,3,4,6,7,8-HeptaCDF Kerkvliet et al. 1985									
1,2,3,4,6,7,8,9-OctaCDF									
30	RAT (Long-Evans) 4–14	4 days (GO)	0.03–90	BW BC	Endocr	300			
1,2,3,4,6,7,8,9-OctaCDF Crofton et al. 2005									
INTERMEDIATE EXPOSURE									
2,3,7,8-TetraCDF									
31	Monkey (Rhesus) 3 M	2 months (F)	2.1	BW GN HP BC CS	Death			2.1	1/3 monkeys died
					Gastro		2.1		Intramucosal cysts
					Hemato	2.1			
					Hepatic		2.1		Altered bile duct epithelium
					Dermal			2.1	Facial and body hair and nail loss, absent sebaceous glands
					Ocular		2.1		Periorbital edema
					Immuno			2.1	Thymic atrophy
2,3,7,8-TetraCDF McNulty et al. 1981									

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
32	Monkey (Rhesus) 3 M	6 months (F)	0.21	BW GN HP BC CS	Death			0.21	1/3 monkeys died
					Gastro		0.21		Metaplasia of gastric mucosa
					Hemato	0.21			
					Dermal		0.21		Partial sebaceous gland atrophy, hyperkeratotic nail beds
					Ocular		0.21		Periorbital edema, meibomian gland enlargement
					Immuno			0.21	Thymic atrophy
2,3,7,8-TetraCDF McNulty et al. 1981									
33	Mouse (C57BL/ 6N) 8 M	30 days 5 days/week (GO)	0, 30, 100, 300	BW GN HP BC CS	Bd wt	300			
					Hemato	100	300		37% decreased total leukocytes
					Immuno		300		Marked decrease in thymus weight
2,3,7,8-TetraCDF Moore et al. 1979									
34	Guinea pig (Hartley) 3–8 F	6 weeks 1 day/week (GO)	0, 0.05, 0.17, 0.5, 1.0	BW OW GP	Death			1	30% mortality
					Bd wt	1			
					Hemato	1			
					Immuno	0.17	0.5		Thymic atrophy, macrophage inhibition
2,3,7,8-TetraCDF Luster et al. 1979a, 1979b									

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
1,2,3,4,8-PentaCDF									
35	Rat (Sprague-Dawley) 6 M, 6 F	13 weeks (F)	0, 60, 600	BW OW GN HP BC CS	Bd wt	600			
					Cardio	600			
					Hemato	600			
					Hepatic	600			
					Renal	600			
					Endocr	600			
					Immuno	600			
					Repro	600			
1,2,3,4,8-PentaCDF **Pluess et al. 1988a (results also reported in Poiger et al. 1989)**									
1,2,3,7,8-PentaCDF									
36	Rat (Sprague-Dawley) 6 M, 6 F	13 weeks (F)	0, 0.2, 2, 20	BW OW GN HP BC	Bd wt	2 F	20 F		11% decreased terminal body weight
					Cardio	20			
					Hemato	20			
					Hepatic	2 M	20 M		Increased liver weight, vacuolization with lipid accumulation, single cell necrosis
					Renal	20			
					Endocr	20			
					Immuno	2[c]	20		Decreased thymus weight ($BMDL_{1SD}$ of 0.68 µg/kg/day)
					Repro	20			
1,2,3,7,8-PentaCDF **Pluess et al. 1988a (results also reported in Poiger et al. 1989)**									

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
2,3,4,7,8-PentaCDF									
37	Rat (Sprague-Dawley) 10 F	14 weeks 5 days/week (GO)	0, 0.006, 0.020, 0.044, 0.092, 0.200	CS BW OW BC HP	Bd wt	0.2			
					Resp	0.2			
					Gastro	0.2			
					Hepatic	0.044	0.092		Hepatocellular hypertrophy
					Endocr	0.02	0.044		Thyroid gland follicular cell hypertrophy; decreased total T4 levels (25%) at 0.092 µg/kg
					Immuno	0.2			
					Repro	0.2			
2,3,4,7,8-PentaCDF NTP 2006									
38	Rat (Sprague-Dawley) 10 F	31 weeks 5 days/week (GO)	0, 0.006, 0.020, 0.044, 0.092, 0.200	CS BW OW BC HP	Bd wt	0.2			
					Resp	0.2			
					Gastro	0.2			
					Hepatic	0.02	0.044		Hepatocellular hypertrophy
					Endocr		0.006[d]		Decreased (16%) total T4 levels; increased total T3 levels at ≥0.092 µg/kg ($BDML_{ADJ}$ of 0.00068 µg/kg/day)
					Immuno	0.092	0.2		Thymic cortical atrophy
					Repro	0.2			
2,3,4,7,8-PentaCDF NTP 2006									

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
39	Rat (Sprague-Dawley) 6 M, 6 F	13 weeks (F)	0, 0.2, 2, 20	BW OW GN HP BC CS	Death			20	92% mortality
					Bd wt	0.2	2	20	LOAEL: 11% decreased body weight gain Serious LOAEL: 47–54% body weight loss
					Cardio	20			
					Hemato	20			
					Hepatic		0.2		Increased serum bilirubin (32–52%), decreased serum triglycerides (males, 18%), slight fatty degeneration in liver
					Renal	20			
					Endocr	20			
					Immuno		0.2 F		LOAEL: decreased thymus weight (24% at 0.2 µg/kg/day and 90% at 2 µg/kg/day)
					Repro	20			
2,3,4,7,8-PentaCDF **Pluess et al. 1988b (results also reported in Poiger et al. 1989)**									
40	MOUSE (B6C3F1) 10–12 F	5 times in a 16-week period (GO)	0, 10, 30, 100	OW HP	Immuno	10	30		Decreased thymus weight (13%)
					Repro	30	100		Enhanced promotion of surgically-induced endometriosis
2,3,4,7,8-PentaCDF **Johnson et al. 1997**									

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
1,2,3,6,7,8-HexaCDF									
41	Rat (Sprague-Dawley) 6 M, 6 F	13 weeks (F)	0, 0.2, 2, 20	BW OW GN HP BC	Bd wt	2	20		14–20% decreased body weight gain
					Cardio	20			
					Hemato	20			
					Hepatic	0.2	2		Increased liver weight, vacuolization with lipid accumulation, single cell necrosis
					Renal	20			
					Endocr	20			
					Immuno	0.2[e]	2		LOAEL: decreased thymus weight (40–42% at 2 µg/kg/day and 75–79% at 20 µg/kg/day) ($BMDL_{1SD}$ of 0.48 µg/kg/day)
					Repro	20			
1,2,3,6,7,8-HexaCDF Pluess et al. 1988a (results also reported in Poiger et al. 1989)									
Mixed CDFs									
42	Rat (Sprague-Dawley) 6 M	4 weeks (F)	0, 50, 500	BI	Hemato			50	Hemolytic anemia
					Hepatic		50		Porphyria
Mixed CDFs Oishi and Hiraga 1978									

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
43	Rat (Sprague-Dawley) 10 M	4 weeks (F)	0, 97, 960	OW GN BC CS	Bd wt		97		15% decreased body weight gain
					Cardio	960			
					Hemato		97		Decreased hemoglobin, hematocrit and MCV, increased MCHC
					Hepatic		97		Increased liver weight and lipid content
					Renal	960			
					Dermal	97		960	Chloracne
					Immuno		97		Decreased thymus weight
					Repro	97	960		Decreased relative seminal vesicle weight and testosterone concentration in testes
Mixed CDFs Oishi et al. 1978									
44	Mouse (ICR) 10 M	4 weeks 1 day/week (GO)	0, 10, 100	OW CS	Immuno	10	100		Decreased thymus weight
Mixed CDFs Oishi and Hiraga 1980									

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
CHRONIC EXPOSURE									
2,3,4,7,8-PentaCDF									
45	Rat (Sprague-Dawley) 80 F	105 weeks 5 days/week (GO)	0, 0.006, 0.020, 0.044, 0.092, 0.200	CS BW OW BC HP	Bd wt	0.2			
					Resp	0.044	0.092		Bronchiolar metaplasia of alveolar epithelium
					Cardio	0.092	0.2		Cardiomyopathy
					Gastro	0.092	0.2		Squamous hyperplasia of the forestomach
					Musc/skel	0.2			
					Hepatic		0.006[f]		Minimal hepatocellular hypertrophy; diffuse fatty changes at ≥0.02 µg/kg; minimal to mild necrosis, bile duct hyperplasia, bile duct fibrosis, and cholangiofibrosis at 0.2 µg/kg
					Renal	0.02	0.044		Nephropathy
					Dermal	0.2			
					Ocular	0.2			
					Endocr		0.006		Cystic degeneration in adrenal cortex at ≥0.006 µg/kg; follicular cell hypertrophy in thyroid gland at ≥0.020 µg/kg; decreased (22%) serum total T4 levels at ≥0.044 µg/kg (measured at 53 weeks), increased (23%) serum total T3 levels at ≥0.092 (measured at 53 weeks); and arterial chronic active inflammation in pancreas at 0.200 µg/kg
					Immuno	0.092	0.2		Increased severity of thymic atrophy

Table 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral (µg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Neuro	0.2			
					Repro	0.02	0.044		Squamous metaplasia in uterus
					Other noncancer	0.02	0.044		Gingival squamous hyperplasia
					Cancer			0.2	Hepatocellular adenoma and cholangiocarcinoma of the liver and gingival squamous cell carcinoma of the oral mucosa
2,3,4,7,8-PentaCDF NTP 2006									

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive an acute-duration oral MRL of 0.0005 µg/kg/day for 2,3,4,7,8-pentaCDF. The NOAEL of 0.5 µg/kg was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability) and modifying factor of 10. See Appendix A for details.
[c]Used to derive an intermediate-duration oral MRL of 0.007 µg/kg/day for 1,2,3,7,8-pentaCDF. The $BMDL_{1SD}$ of 0.68 µg/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.
[d]Used to derive an intermediate-duration oral MRL of 0.000007 µg/kg/day for 2,3,4,7,8-pentaCDF. The $BMDL_{1SD}$ of 0.00095 µg/kg/day was adjusted to continuous exposure to a $BMDL_{ADJ}$ of 0.00068 µg/kg/day and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability). See Appendix A for details.
[e]Used to derive an intermediate-duration oral MRL of 0.005 µg/kg/day for 1,2,3,6,7,8-hexaCDF. The $BMDL_{1SD}$ of 0.48 µg/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.
[f]Used to derive a chronic-duration oral MRL of 0.000004 µg/kg/day for 2,3,4,7,8-pentaCDF. The LOAEL of 0.006 µg/kg/day adjusted to continuous exposure to a $LOAEL_{ADJ}$ of 0.0042 µg/kg/day and divided by an uncertainty factor of 1,000 (10 for the use of a LOAEL, 10 for extrapolation from animals to humans and 10 for human variability). See Appendix A for details.

ADJ = adjusted; BC = blood chemistry; Bd wt or BW = body weight; BI = biochemical changes; BMDL = 95% lower confidence limit on the benchmark dose; BUN = blood urea nitrogen; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental toxicity; ED_{50} = 50% effective dose; Endocr = endocrine; (F) = feed; F = female(s); FX = fetal toxicity; (G) = gavage; (GO) = gavage in oil; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; Hemato = hematological; HP = histopathology; Immuno = immunological; IX = immune function; LOAEL = lowest-observed-adverse-effect level; M = male(s); MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MRL = Minimal Risk Level; Musc/skel = muscular/skeletal; MX = maternal toxicity; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OW = organ weight; PND = postnatal day; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; SD = standard deviation; SRBC = sheep red blood cell; T4 = thyroxine; UR = urinalysis

Principal study for an MRL.

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

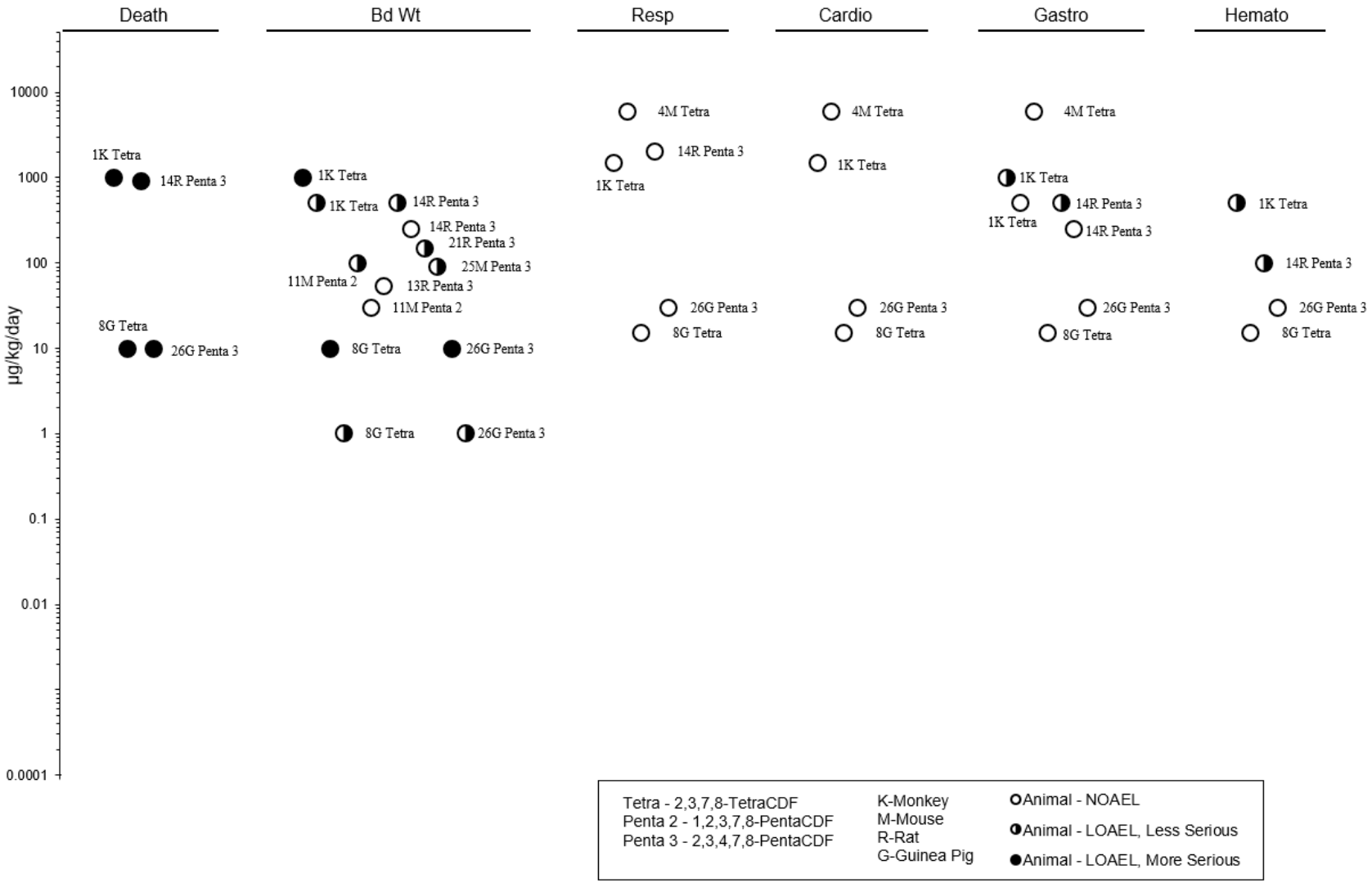


Figure 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral
Acute (≤14 days)

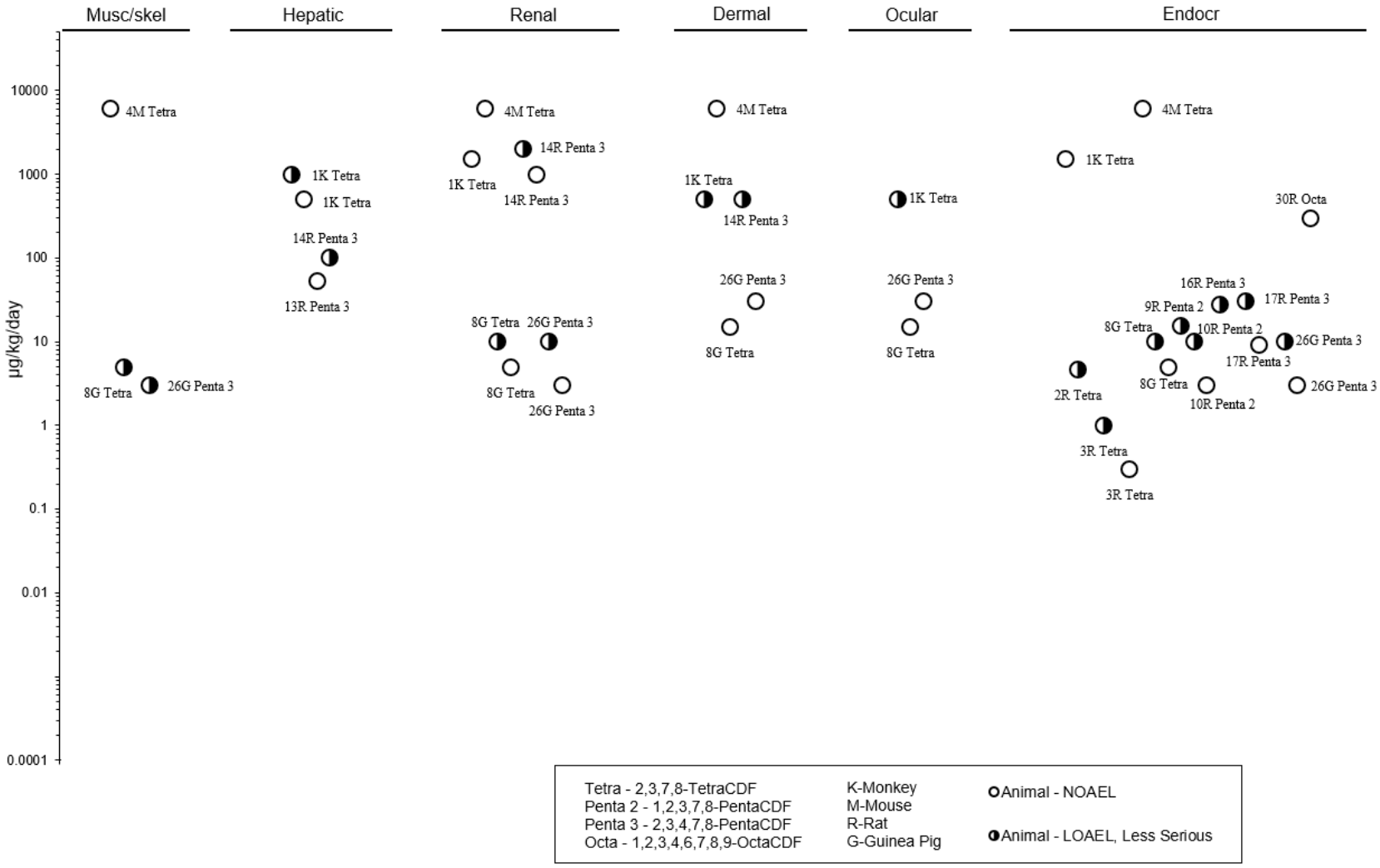


Figure 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral
Acute (≤14 days)

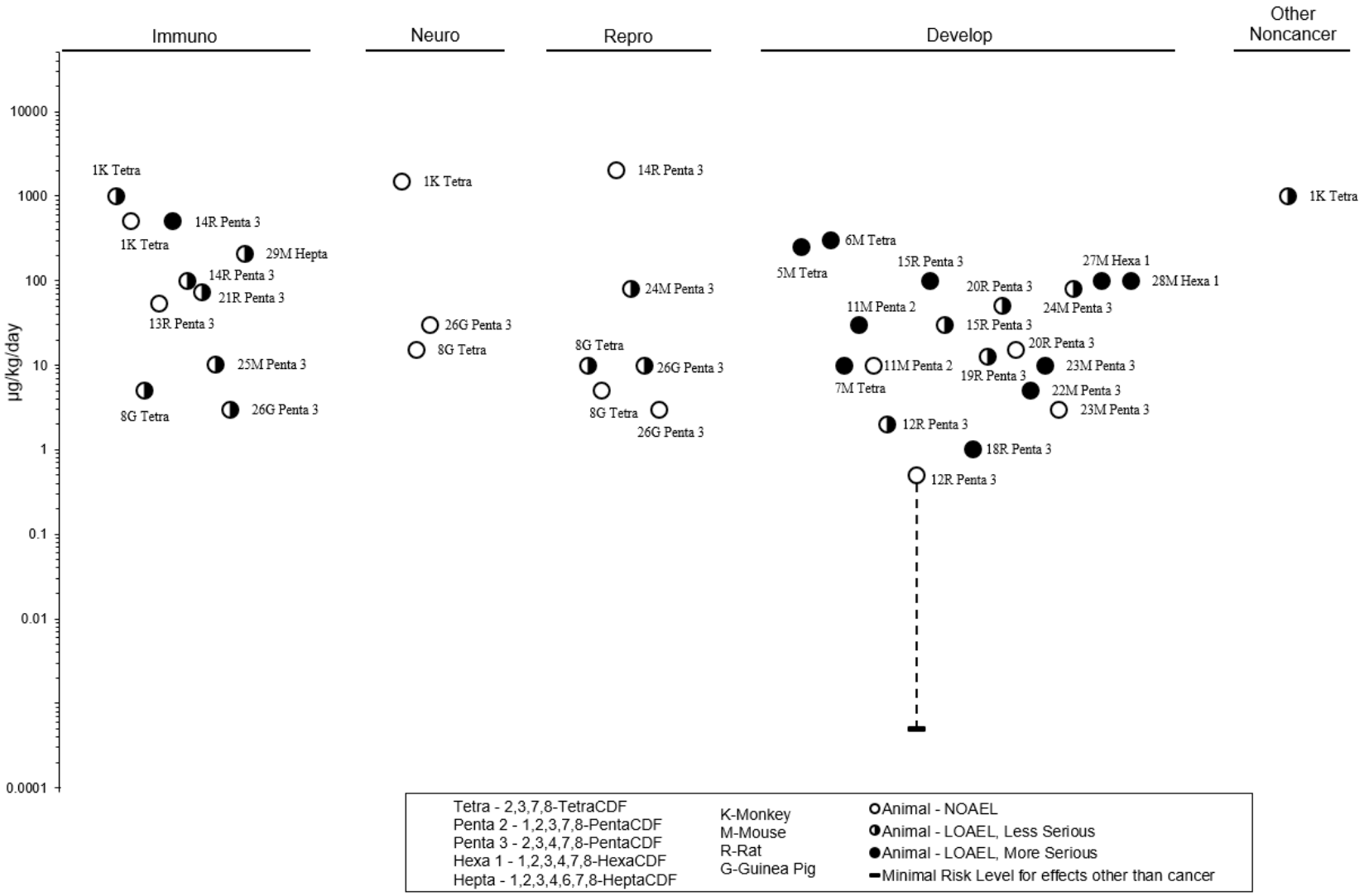


Figure 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral
Intermediate (15–364 days)

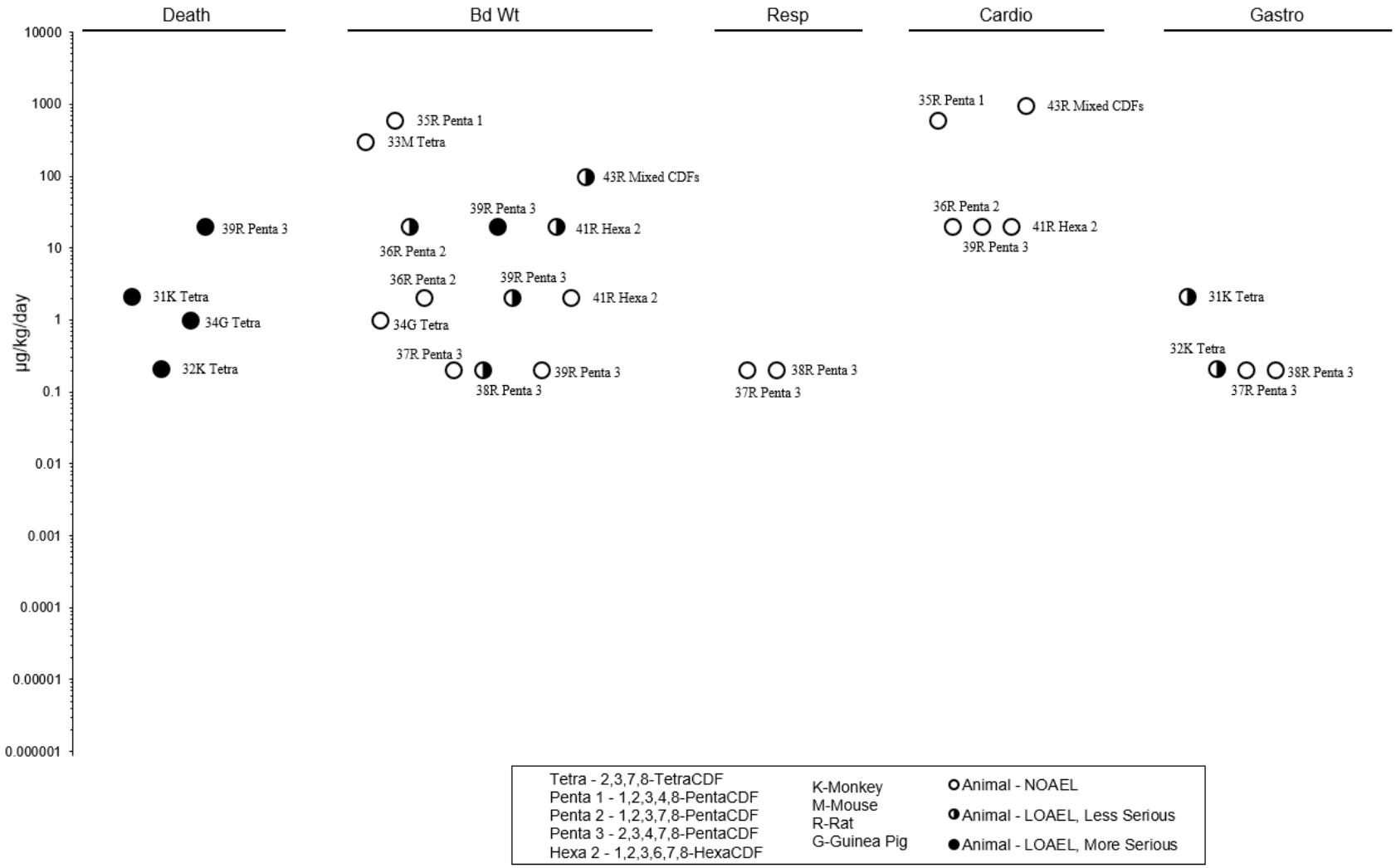


Figure 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral
Intermediate (15–364 days)

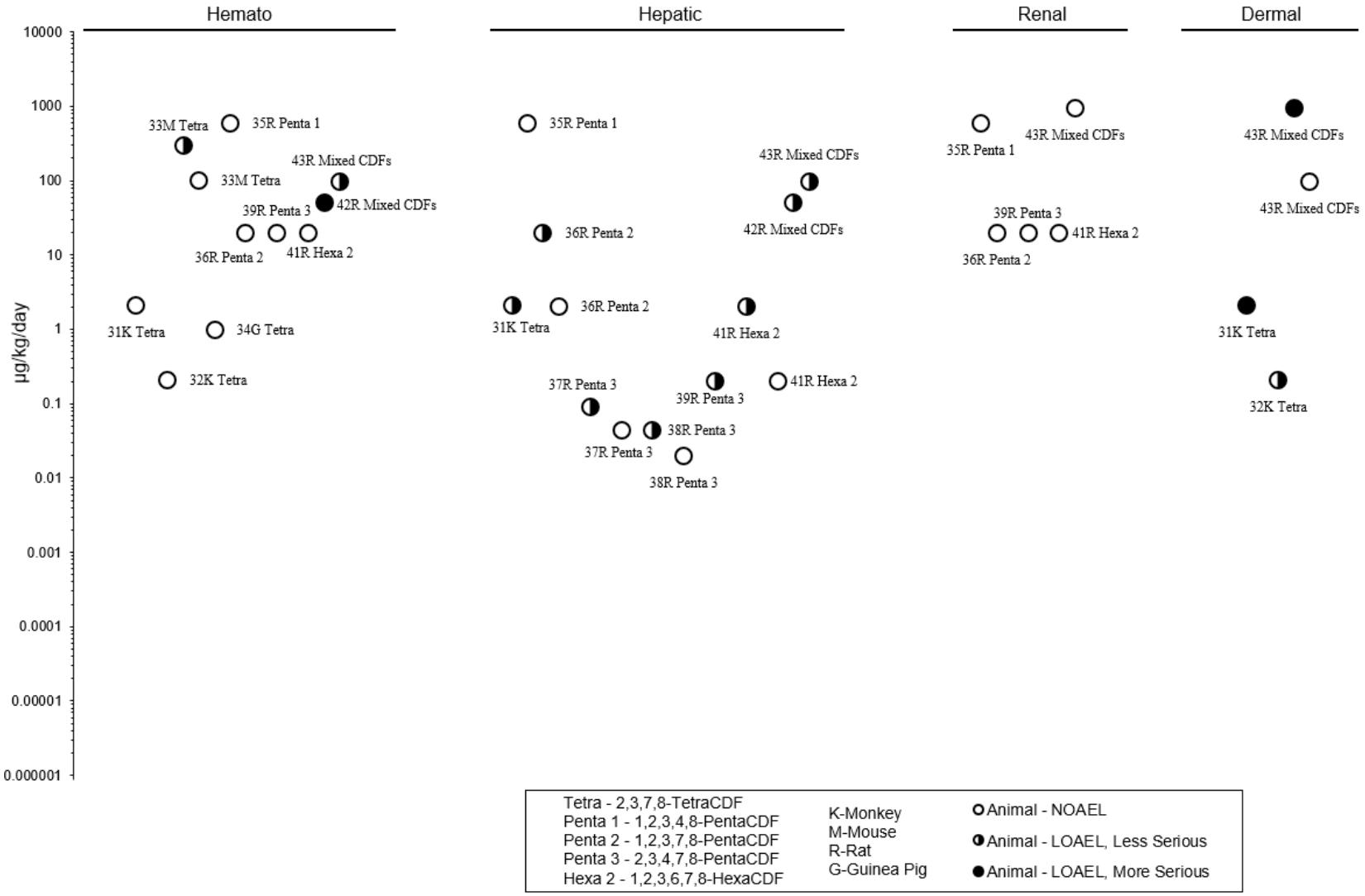


Figure 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral
Intermediate (15–364 days)

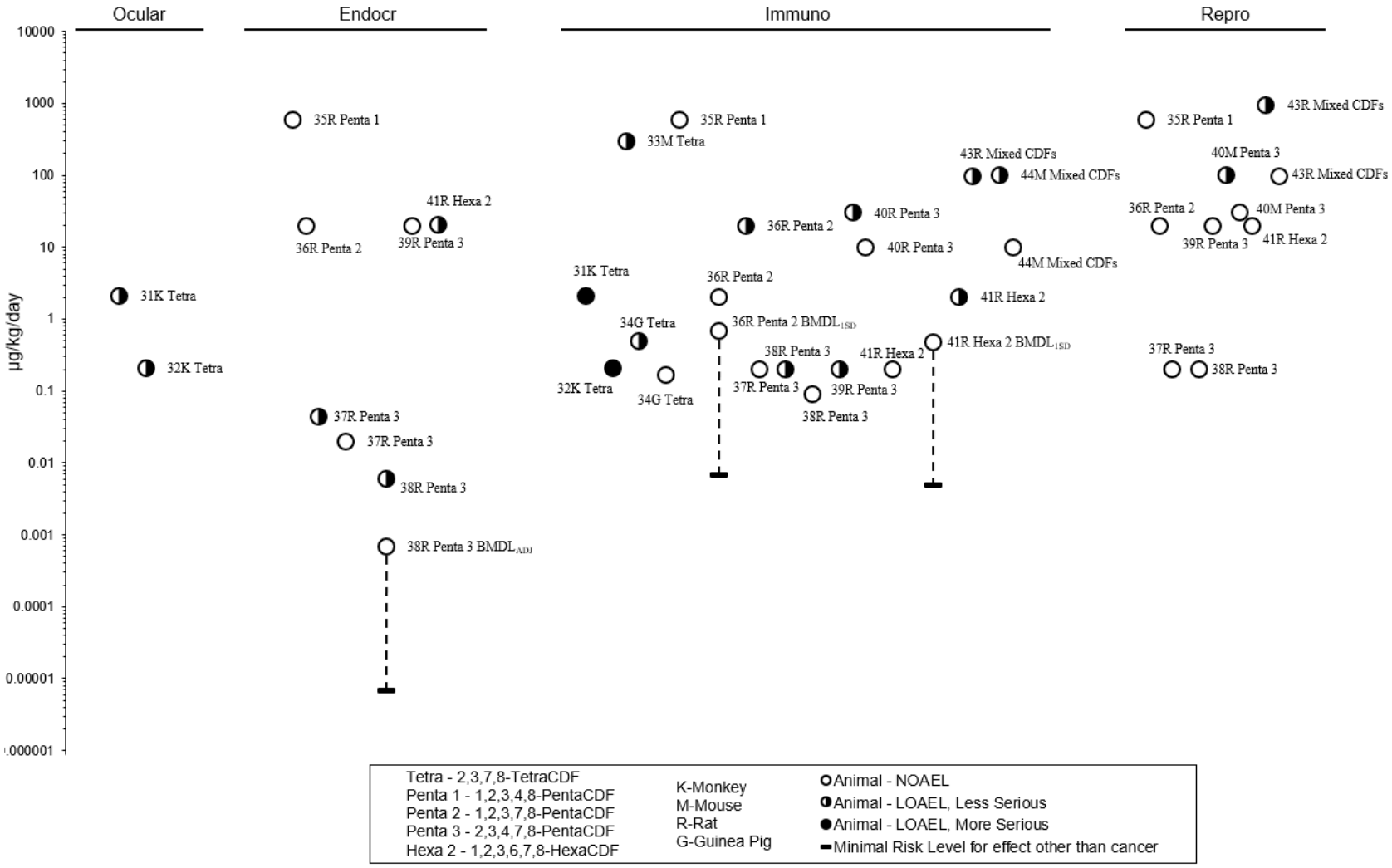


Figure 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral
Chronic (≥365 days)

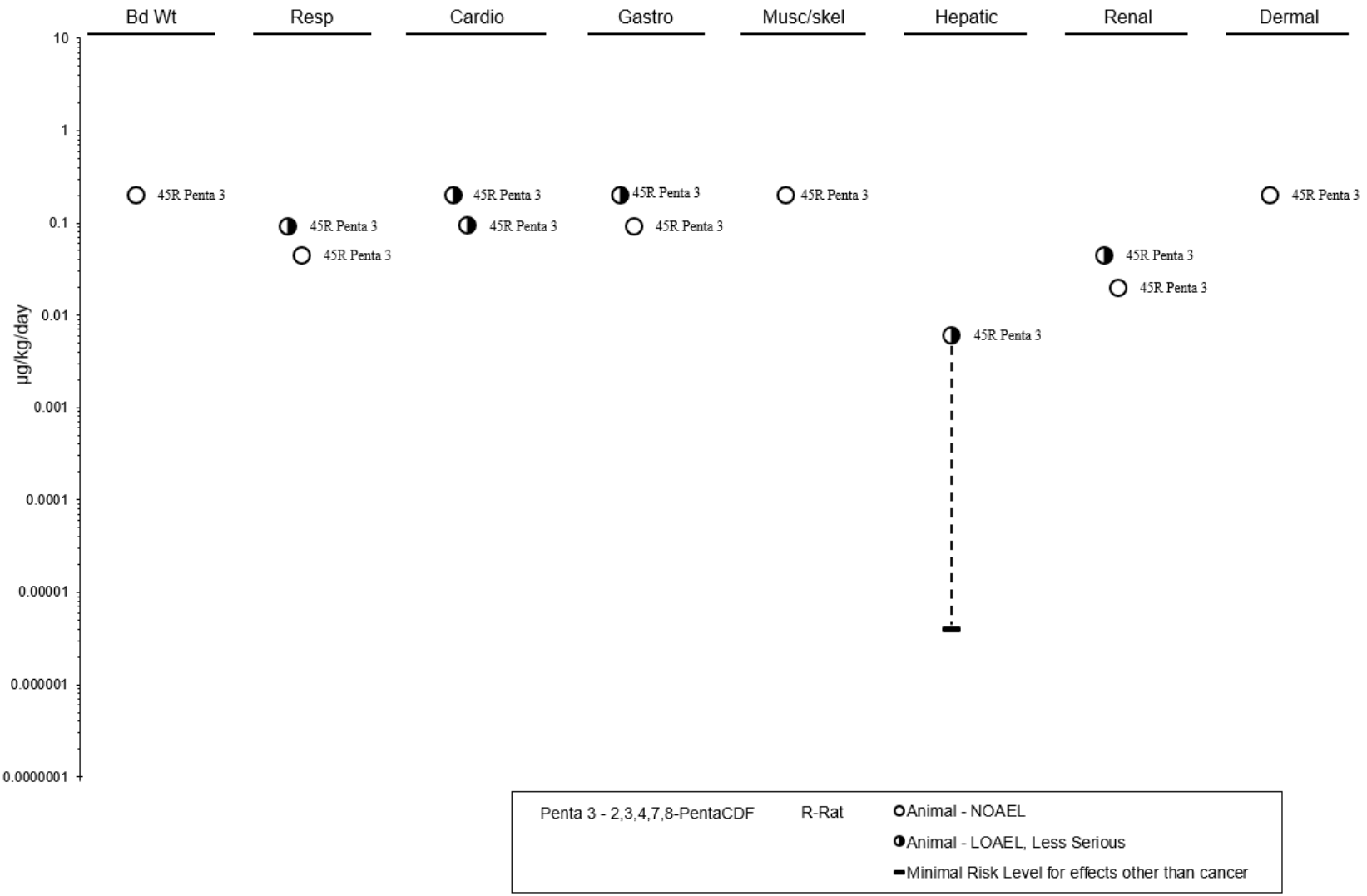


Figure 2-2. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Oral
Chronic (≥365 days)

Table 2-3. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Dermal

Species (strain) No./group	Exposure parameters	Doses (µg/kg/day)	Parameters monitored	Endpoint	NOAEL (µg/kg/day)	Less serious LOAEL (µg/kg/day)	Serious LOAEL (µg/kg/day)	Effects
INTERMEDIATE EXPOSURE								
Mouse (HRS/J) 20 F	20 weeks 2 days/week	0, 33.3	GN	Cancer			33.3 F	CEL: skin papillomas following initiation
Comment: Mice were treated with a single 5 µmol dermal dose of MNNG in acetone prior to exposure to 2,3,7,8-tetraCDF.								
2,3,7,8-TetraCDF **Poland et al. 1982**								
Mouse (HRS/J) 20 F	20 weeks 2 days/week	0, 3.3	BW OW HP CS	Bd wt		3.3 F		12% decreased body weight gain
				Gastro	3.3 F			
				Hepatic		3.3 F		Increased liver weight and hypertrophy
				Immuno			3.3 F	Thymic and splenic lymphoid atrophy
Comment: Mice were treated with a single dermal dose of acetone prior to exposure to 2,3,4,7,8-pentaCDF.								
2,3,4,7,8-PentaCDF **Hebert et al. 1990**								
Mouse (HRS/J) 20 F	20 weeks 2 days/week	0, 0.08, 1.7 or 3.3	BW OW HP CS	Cancer			0.08 F	CEL: skin proliferative lesions following initiation
Comment: Mice were treated with a single 5 µmol dermal dose of MNNG in acetone prior to exposure to 2,3,4,7,8-pentaCDF.								
2,3,4,7,8-PentaCDF **Hebert et al. 1990**								
Mouse (HRS/J) 20 F	20 weeks 2 days/week	0, 3.3	HP	Death			3.3 F	35% mortality
				Bd wt		3.3 F		8% body weight loss
				Gastro		3.3 F		Mucous cell hyperplasia of glandular stomach
				Hepatic		3.3 F		Increased liver weight and hypertrophy

Table 2-3. Levels of Significant Exposure to Chlorodibenzofurans (CDFs) – Dermal

Species (strain) No./group	Exposure parameters	Doses (µg/kg/day)	Parameters monitored	Endpoint	NOAEL (µg/kg/day)	Less serious LOAEL (µg/kg/day)	Serious LOAEL (µg/kg/day)	Effects
				Immuno			3.3 F	Thymic and splenic lymphoid atrophy
Comment: Mice were treated with a single dermal dose of acetone prior to exposure to 1,2,3,4,7,8-hexaCDF.								
1,2,3,4,7,8-HexaCDF **Hebert et al. 1990**								
Mouse (HRS/J) 20 F	20 weeks 2 days/week	0, 8.3, 16.7, 33.3	BW OW HP CS	Death			33.3 F	Increased mortality
				Cancer			8.3 F	CEL: skin proliferative lesions following initiation
Comment: Mice were treated with a single 5 µmol dermal dose of MNNG in acetone prior to exposure to 1,2,3,4,7,8-hexaCDF.								
1,2,3,4,7,8-HexaCDF **Hebert et al. 1990**								

Bd wt or BW = body weight; CEL = Cancer Effect Level; CS = clinical signs; F = female(s); Gastro = gastrointestinal; HP = histopathology; Immuno = immunological; LOAEL = lowest-observed-adverse-effect level; MNNG = methylnitronitrosoguanidine; NOAEL = no-observed-adverse-effect level; OW = organ weight

2.2 DEATH

Several studies evaluated mortality among Yusho and Yu-Cheng victims. No increases in deaths from all causes were observed when Yusho victims were followed through 1983 (Kuratsune et al. 1987), 2007 (Onozuka et al. 2009), or 2017 (Onozuka et al. 2020). Similarly, studies of the Yu-Cheng victims did not demonstrate increases in deaths from all causes when victims were followed through 1991 (Yu et al. 1997) or 2003 (Tsai et al. 2007). However, in a study that used neighborhood referents rather than national referents, an increase in the prevalence of deaths from all causes was found among Yu-Cheng victims followed through 2008 (Li et al. 2013). A meta-analysis of the results of the Onozuka et al. (2009) and Li et al. (2013) studies of Yusho and Yu-Cheng victims reported an increase in deaths from all causes (standardized mortality ratio [SMR] 1.1, 95% confidence interval (CI) 1.1–1.2) (Li et al. 2015a). Studies also reported cause-specific deaths, which are discussed in subsequent sections of this chapter.

Information on lethality of CDFs in animals following acute oral exposure is available for several congeners. Due to a long latent period for the onset of toxicity, reliable determination of toxic dose following acute exposure requires a sufficient observation period (typically 30 days in rodents).

2,3,7,8-TetraCDF. Single 2,3,7,8-tetraCDF doses ≥1,000 µg/kg, but not 500 µg/kg, were lethal in rhesus monkeys observed for 60 days post-exposure, but small numbers of animals (two to four) were tested (Moore et al. 1979). A CDF mixture containing 88% 2,3,7,8-tetraCDF (remainder primarily an unidentified pentaCDF) did not cause death in mice when tested at doses ≤6,000 µg/kg (Moore et al. 1976, 1979). The Hartley guinea pig was the most sensitive of the species tested as indicated by lethality following single doses of 10 µg/kg 2,3,7,8-tetraCDF (Moore et al. 1979).

Although limited by small numbers of animals (three to eight per dosage), gavage studies with 2,3,7,8-tetraCDF indicate that Hartley guinea pigs are much more sensitive than C57B1/6Fh mice following repeated exposure (Ioannou et al. 1983; Moore et al. 1979). Weekly doses of 1 µg/kg for 6–14 weeks produced 30–70% mortality in guinea pigs, whereas 22 doses of 300 µg/kg in 30 days caused no deaths in mice observed for an additional 30 days (Luster et al. 1979a, 1979b). One of three monkeys died following dietary administration of 2,3,7,8-tetraCDF in estimated dosages of 2.1 µg/kg/day for 2 months or 0.21 µg/kg/day for 6 months (McNulty et al. 1981).

1,2,3,4,8-PentaCDF. No deaths were observed in Sprague-Dawley rats exposed to ≤600 µg/kg/day in the diet for 13 weeks (Pluess et al. 1988a).

1,2,3,7,8-PentaCDF. Dietary exposure to doses as high as 20 µg/kg/day did not result in deaths in Sprague-Dawley rats exposed for 13 weeks (Pluess et al. 1988a).

2,3,4,7,8-PentaCDF. An LD_{50} of 916 µg/kg was estimated for 2,3,4,7,8-pentaCDF in male rats (Brewster et al. 1988). Lethality was observed in Hartley guinea pigs administered a single gavage dose of 10 µg/kg 2,3,4,7,8-pentaCDF (Moore et al. 1979).

Dietary administration of 20 µg/kg/day 2,3,4,7,8-pentaCDF for 13 weeks caused >90% mortality in rats (Pluess et al. 1988b). No deaths were observed in female rats administered via gavage ≤0.2 µg/kg 2,3,4,7,8-pentaCDF 5 days/week for 2 years (NTP 2006).

1,2,3,6,7,8-HexaCDF. No deaths were observed in a 13-week study of rats exposed to ≤20 µg/kg/day 1,2,3,6,7,8-hexaCDF in the diet (Pluess et al. 1988a).

Summary. The existing lethality data indicate that congeners substituted in the 2,3,7,8-positions, particularly 2,3,4,7,8-pentaCDF and 2,3,7,8-tetraCDF, are the most toxic congeners tested. There is a marked species variation in sensitivity, with the guinea pig and monkey being particularly sensitive, although this may differ for other endpoints. Single doses as low as 10 µg/kg and repeated doses as low as 0.21 µg/kg/day for 6 months were extremely toxic, causing death in guinea pigs and monkeys, respectively. A wasting syndrome was the major toxic effect at lethal doses in most species (see Section 2.3), but this may not be the only cause of death.

2.3 BODY WEIGHT

Several epidemiological studies evaluated body weight endpoints (see Table 2-4). A study of Yusho victims reported an association between serum 2,3,4,7,8-pentaCDF levels and body weight loss (Imamura et al. 2007). In a study of residents living near a highly dioxin-contaminated site in Taiwan, abdominal obesity, defined as a waist to hip ratio of greater than 0.8 in women and 0.9 in men, was associated with serum concentrations of several tetra-, penta-, and hexaCDF congeners in men and with some heptaCDF congeners in females (Chang et al. 2016). In a study of the general population, no association between total CDF TEQ levels and the risk of having a body mass index (BMI) of ≥25 kg/m^2 was found (Uemura et al. 2009), while a similar study found no association between CDF concentrations in fat and BMI in patients undergoing gastric bypass or abdominal surgery (Deshmukh et al. 2020).

Table 2-4. Results of Epidemiological Studies Evaluating Exposure to CDFs and Body Weight Effects

Reference, study type, and population	Biomarker[a]	Outcome evaluated	Result
Chang et al. 2016 Cross-sectional; 2,876 adults living near a highly dioxin-contaminated site (Taiwan)	Serum 2,3,7,8-tetraCDF TEQ (levels not reported)	Abdominal obesity	↑ 3Q (males) ↔ (females)
	Serum 1,2,3,7,8-pentaCDF TEQ (levels not reported)	Abdominal obesity	↑ 2Q (males) ↔ (females)
	Serum 2,3,4,7,8-pentaCDF TEQ (levels not reported)	Abdominal obesity	↑ 3Q (males) ↔ (females)
	Serum 1,2,3,4,7,8-hexaCDF TEQ (levels not reported)	Abdominal obesity	↑ 2Q (males) ↔ (females)
	Serum 1,2,3,6,7,8-hexaCDF TEQ (levels not reported)	Abdominal obesity	↑ 3Q (males) ↔ (females)
	Serum 2,3,4,6,7,8-hexaCDF TEQ (levels not reported)	Abdominal obesity	↑ 3Q (males) ↑ 3Q (females)
	Serum 1,2,3,7,8,9-hexaCDF TEQ (levels not reported)	Abdominal obesity	↔ (males) ↔ (females)
	Serum 1,2,3,4,6,7,8-heptaCDF TEQ (levels not reported)	Abdominal obesity	↔ (males) ↑ 4Q (females)
	Serum 1,2,3,4,7,8,9-heptaCDF TEQ (levels not reported)	Abdominal obesity	↔ (males) ↑ 3Q (females)
	Serum octaCDF TEQ (levels not reported)	Abdominal obesity	↔ (males) ↔ (females)
	Serum total CDF TEQ (levels not reported)	Abdominal obesity	↑ 2Q (males) ↔ (females)
Deshmukh et al. 2020 Cross-sectional, 106 patients undergoing gastric bypass or abdominal surgery (United Kingdom)	10 CDFs in subcutaneous fat, visceral fat and liver (levels not reported)	BMI	↔
Imamura et al. 2007 Retrospective, 241 adults in Yusho cohort	Serum total CDF mean of 264.26 pg/g lipid	Body weight	↑
Uemura et al. 2009 Cross-sectional, 1,374 adults (Japan)	Serum total CDF TEQ 4th quartile ≥6.60 pg/g lipid	BMI ≥25 kg/m^2	↔

[a]TEQs were calculated using the WHO 1998 TEF values (van den Berg et al. 1998).

↑ = association between biomarker level and outcome; ↓ = inverse association between biomarker level and outcome; ↔ = no association between biomarker level and outcome; BMI = body mass index; CDF = chlorodibenzofuran; Q = quartile; TEF = toxic equivalency factor; TEQ = toxic equivalent; WHO = World Health Organization

2,3,7,8-TetraCDF. A single gavage dose caused wasting effects in guinea pigs at ≥10 µg/kg 2,3,7,8-tetraCDF (Moore et al. 1979) and monkeys at 1,000 µg/kg 2,3,7,8-tetraCDF (Moore et al. 1979), as evidenced by rapid and progressive weight loss. Decreases in weight gain were also observed in monkeys administered 500 µg/kg and guinea pigs administered 1 µg/kg 2,3,7,8-tetraCDF (Moore et al. 1979). Decreased body weight gain was observed following a single dose of 10,000 µg/kg (GD 15) in pregnant female SD rats in the 3–4 days following treatment (16–74%) and overall (16–26%), although no changes were observed in the repeated dose (GD 8–10) studies (Johnson et al. 2020). No alterations in body weight gain were observed in intermediate-duration studies in which mice were administered 300 µg/kg 5 days/week for 30 days (Moore et al. 1979) or in guinea pigs administered 1 µg/kg 1 day/week for 6 weeks (Luster et al. 1979a, 1979b).

1,2,3,4,8-PentaCDF. No alterations in body weight gain were observed in rats exposed to 600 µg/kg/day in the diet for 13 weeks (Pluess et al. 1988a).

1,2,3,7,8-PentaCDF. A 6.5–11% decrease in body weight gain was observed in rats exposed to 20 µg/kg/day 1,2,3,7,8-pentaCDF in the diet for 13 weeks (Pluess et al. 1988a); no alterations were observed at 2 µg/kg/day.

2,3,4,7,8-PentaCDF. Wasting effects were observed in guinea pigs administered a lethal gavage dose of 10 µg/kg (Moore et al. 1979). At lower concentrations (≥1 µg/kg), decreases in body weight gain were observed. Rats appear to be less sensitive to the body weight effects of 2,3,4,7,8-pentaCDF. A decrease in body weight gain was observed in rats administered a single dose of 500 µg/kg (Brewster et al. 1988). An ED_{50} (50% decrease in body weight gain) of 146 µg/kg was estimated in pubertal rats administered a single dose of 2,3,4,7,8-pentaCDF (Taura et al. 2014). Single dose studies of 2,3,4,7,8-pentaCDF did not result in alterations in body weight in rats administered 53 µg/kg (Ahlborg et al. 1989) or in mice administered 90 µg/kg (Johnson et al. 2000).

In intermediate-duration studies, body weight gain was decreased (11%) in rats fed 2 µg/kg/day dosages of 2,3,4,7,8-pentaCDF and weight loss (47–50%) was observed at 20 µg/kg/day dosage (Pluess et al. 1988b). No alterations in body weight gain were observed in rats administered 0.2 µg/kg 5 days/week (NTP 2006). In the only available chronic study, no alterations in body weight gain were observed in female rats administered ≤0.2 µg/kg 2,3,4,7,8-pentaCDF 5 days/week for 2 years (NTP 2006).

Mixed Chlorodibenzofurans. Body weight gain was decreased in rats exposed to ≥97 µg/kg/day of an uncharacterized mixture of two tetra-, four penta-, and four hexaCDFs in the diet for 4 weeks (Oishi et al. 1978).

Summary. Studies in laboratory animals reported decreases in body weight gain at lower doses and a wasting syndrome in animals exposed to high, often lethal, doses. The wasting syndrome is characterized by progressive decreased weight gain, with immediate moderate to severe body weight loss generally occurring at near-lethal doses.

2.4 RESPIRATORY

Clinical observations strongly suggest that Yusho and Yu-Cheng cohort members experienced frequent or more severe respiratory infections (Akahane et al. 2018; Kuratsune 1989; Rogan 1989). Chronic bronchitis accompanied by persistent cough and sputum production was observed in 40–50% of some examined patients, with symptoms gradually improving during 5–10 years following onset (Nakanishi et al. 1985; Shigematsu et al. 1971, 1977). Physical findings differed from those in usual bronchitis in that many nonsmokers showed no crackles and some showed wheezes without radiologic, physiologic, or immunologic evidence of bronchial asthma or pulmonary emphysema (Nakanishi et al. 1985; Shigematsu et al. 1971). Kanagawa et al. (2008) found an association between serum levels of 2,3,4,7,8-pentaCDF and abnormal respiratory sounds in a retrospective study of 501 Yusho victims. A mortality study of the Yu-Cheng cohort did not find increases in the risk of death from respiratory disease (examination period was 1980–2003) (Tsai et al. 2007).

A cohort study evaluated the association between lung function and dioxin exposure in foundry workers compared to nonexposed general residents in area of central China in 2013 (Zhang et al. 2020). In general, foundry workers had significantly lower forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) levels and higher levels of urinary oxidative stress biomarkers than the residents. Examination of all participants revealed associations between CDF exposure and decreased lung function regardless of smoking status. In a categorical analysis using quartiles of total serum CDFs, the highest quartile had lower FEV_1 and FVC than the lowest quartile.

2,3,7,8-TetraCDF. A single dose exposure did not result in histological alterations in the trachea or lungs of monkeys administered ≤1,500 µg/kg, mice administered ≤6,000 µg/kg, or guinea pigs administered ≤15 µg/kg 2,3,7,8-tetraCDF (Moore et al. 1976, 1979); these dose levels resulted in deaths of monkeys and guinea pigs.

2,3,4,7,8-PentaCDF. No histological alterations were observed in the respiratory tract of rats receiving a single dose of 2,000 µg/kg 2,3,4,7,8-pentaCDF (Brewster et al. 1988) or guinea pigs administered a single dose of 30 µg/kg (Moore et al. 1979). Intermediate-duration studies conducted by the National Toxicology Program (NTP 2006) did not report respiratory lesions in rats administered ≤0.2 µg/kg 2,3,4,7,8-pentaCDF 5 days/week for 14 or 31 weeks. In a chronic-duration study by this group, bronchiolar metaplasia of the alveolar epithelium was observed in rats administered ≥0.092 µg/kg for 2 years (NTP 2006).

Summary. The Yusho and Yu-Cheng data provide evidence that CDFs induced bronchitis and related respiratory effects in humans. There is no evidence of pulmonary histological changes in animals exposed to single doses of CDFs or following intermediate-duration exposure; lung damage was evident after chronic exposure to 2,3,4,7,8-pentaCDF.

2.5 CARDIOVASCULAR

Cardiovascular endpoints were examined in the Yusho and Yu-Cheng cohorts and a general population study; studies evaluating the possible association between CDF levels and health outcomes (Imamura et al. 2007; Kondo et al. 2018; Uemura et al. 2009) are summarized in Table 2-5. Mixed results were found in lethality studies. No increases in death from circulatory disease were found in two studies of the Yu-Cheng cohort examining 1979–1991 (Yu et al. 1997) and 1980–2003 (Tsai et al. 2007) periods; no increases in deaths from heart disease, hypertension, or cerebrovascular disease were found in the Yusho cohort examined through 2007 (Onozuka et al. 2009) and 2017 (Onozuka et al. 2020). In contrast, Li et al. (2013) reported an increase in the prevalence of deaths from circulatory system diseases, other forms of heart disease, cardiac dysrhythmias, and late effects of cerebrovascular disease among Yu-Cheng females and myocardial infarctions among Yu-Cheng males. A meta-analysis of the results from the Li et al. (2013) Yu-Cheng study and the Onozuka et al. (2009) Yusho study found an increased risk of deaths from heart disease (SMR 1.3, 95%CI 1.0–1.7) (Li et al. 2015a).

Table 2-5. Results of Epidemiological Studies Evaluating Exposure to CDFs and Cardiovascular Effects

Reference, study type, and population	Biomarker[a]	Outcome evaluated	Result
Imamura et al. 2007 Retrospective, 241 adults in Yusho cohort (Japan)	Serum total CDF mean of 264.26 pg/g lipid	Blood pressure	↔
		Heart rate	↔
		EKG	↔
Kondo et al. 2018 Retrospective, 140 adults in Yusho cohort (Japan)	Serum 2,3,4,7,8-pentaCDF 4th quartile ≥72.27 pg/g lipid	Hypertension	↑
Uemura et al. 2009 Cross-sectional, 1,374 adults (Japan)	Serum total CDF TEQ 4th quartile ≥6.60 pg/g lipid	High blood pressure[b]	↑

[a]TEQs were calculated using the WHO 1998 TEF values (van den Berg et al. 1998).
[b]High blood pressure defined as ≥130/85 or physician-diagnosed hypertension.

↑ = association between biomarker level and outcome; ↓ = inverse association between biomarker level and outcome; ↔ = no association between biomarker level and outcome; CDF = chlorodibenzofuran; EKG = electrocardiogram; TEF = toxic equivalency factor; TEQ = toxic equivalent; WHO = World Health Organization

Among living members of the Yusho cohort, Akahane et al. (2018) found an increased prevalence of enlarged heart, abnormal cardiac rhythm, fast pulse, palpitations, low blood pressure, and arterial sclerosis. Two other studies found suggestive evidence of cardiovascular effects among cohort members with higher CDF exposures. Kondo et al. (2018) reported an association between high serum 2,3,4,7,8-pentaCDF levels (≥72.27 pg/g lipid) and hypertension in the Yusho cohort, and Wang et al. (2008) found an increased risk of hypertension and cardiovascular disease among Yu-Cheng women who had chloracne. Imamura et al. (2007) did not find an association between serum 2,3,4,7,8-pentaCDF and blood pressure, heart rate, or abnormal electrocardiogram (EKG) findings in the Yusho cohort. A general population study of adults in Japan found an association between serum total CDF TEQ levels and high blood pressure (Uemura et al. 2009).

2,3,7,8-TetraCDF. Administration of a single dose of 2,3,7,8-tetraCDF did not result in histological alterations in the heart of monkeys (≤1,500 µg/kg), mice (≤6,000 µg/kg), or guinea pigs (≤15 µg/kg) (Moore et al. 1976, 1979); the animals were examined at death or following a 30-day (mice and guinea pigs) or 60-day (monkey) observation period.

1,2,3,7,8-PentaCDF. Dietary exposure of rats to 600 µg/kg/day 1,2,3,7,8-pentaCDF for 13 weeks did not result in histological alterations in the heart (Pluess et al. 1988a).

2,3,4,7,8-PentaCDF. No histological alterations were observed in the heart of guinea pigs that were administered a single gavage dose of ≤30 µg/kg 2,3,4,7,8-pentaCDF (Moore et al. 1979). In intermediate-duration studies, no heart histological alterations were observed in rats exposed to 20 µg/kg/day in the diet for 13 weeks (Pluess et al. 1988a) or in rats administered ≤0.2 µg/kg 2,3,4,7,8-pentaCDF 5 days/week for 14 or 31 weeks (NTP 2006). Cardiomyopathy was observed in rats administered 0.2 µg/kg 2,3,4,7,8-pentaCDF 5 days/week for 2 years (NTP 2006).

1,2,3,6,7,8-HexaCDF. Dietary exposure to 20 µg/kg/day 1,2,3,6,7,8-hexaCDF for 13 weeks did not result in histological alterations in rats (Pluess et al. 1988a).

Mixed CDF Congeners. Dietary exposure to an uncharacterized mixture of two tetra-, four penta-, and four hexaCDFs for 4 weeks caused increased relative heart weight at ≥97 µg/kg/day and decreased absolute heart weight at 960 µg/kg/day in rats, but histology was not evaluated (Oishi et al. 1978). The increased relative heart weight is likely due to concurrent lower body weight (see Section 2.3).

Summary. Studies of the Yusho and Yu-Cheng cohorts provide suggestive evidence of an increased risk of heart disease, but the results are not consistent across studies. The results of animal studies with 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, and 1,2,3,6,7,8-hexaCDF suggest that less-than-lifetime exposure does not result in histological alterations in the heart; however, a 2-year study in rats administered 2,3,4,7,8-pentaCDF found altered cardiac histology. The effects of CDFs on the cardiovascular system have not been fully evaluated.

2.6 GASTROINTESTINAL

Early symptoms in Yusho cohort members included vomiting (23.6 and 28% frequencies) and diarrhea (19.1 and 17%) (Kuratsune 1989). Decades after the Yusho incident, Akahane et al. (2018) found an increased prevalence of colon polyps, gastric ulcer, intestinal obstruction, and constipation; Imamura et al. (2007) found an association between serum 2,3,4,7,8-pentaCDF and the frequency of constipation.

2,3,7,8-TetraCDF. Available studies in laboratory animals suggest that the gastrointestinal tract may be a target of 2,3,7,8-tetraCDF toxicity, although there were differences across species. A single gavage dose of 1,000 µg/kg resulted in gastric lesions in rhesus monkeys that were administered a lethal dose of 1,000 µg/kg 2,3,7,8-tetraCDF and observed for 60 days, but not at a nonlethal dose of 500 µg/kg (Moore et al. 1979). Effects including hyperemia, scattered petechial hemorrhage, focal ulceration, and mucosal cysts in the fundic and duodenal areas of the stomach and the small intestine occurred in three of six monkeys. Intermediate-duration exposure also resulted in gastric mucosal changes in rhesus monkeys treated with dietary 2,3,7,8-tetraCDF for 2 or 6 months (McNulty et al. 1981). Mucous metaplasia of the gastric mucosa was found in a monkey that died from ingestion of 0.21 µg/kg/day for 6 months. Intramucosal cysts and cystic growth of mucous glands in the submucosa occurred in the stomach of another monkey that died from ingestion of 2.1 µg/kg/day for 2 months. Although only one animal per dosage was evaluated, these findings are consistent with those observed in the acute study with monkeys and are considered to be exposure-related.

Unlike the monkeys, no histological alterations were observed in the esophagus, stomach, or intestine of mice or guinea pigs administered a single gavage dose of ≤6,000 or 15 µg/kg 2,3,7,8-tetraCDF, respectively, and examined 30 days post-exposure (Moore et al. 1976, 1979).

2,3,4,7,8-PentaCDF. Epithelial hyperplasia of the nonglandular stomach, characterized by acanthosis and hyperkeratosis, was observed in 344 rats that were administered a single, near-lethal, gavage dose of 500 µg/kg 2,3,4,7,8-pentaCDF and observed for 35 days, but not at doses of ≤250 µg/kg (Brewster et al. 1988). In contrast, single gavage dose administration of ≤30 µg/kg 2,3,4,7,8-pentaCDF did not result in histological alterations in the esophagus, stomach, or intestine of guinea pigs examined at the time of death or 30 days post-exposure (Moore et al. 1979). No gastrointestinal lesions were observed in rats administered ≤0.2 µg/kg 5 days/week for 14 or 31 weeks (NTP 2006). However, squamous hyperplasia of the forestomach was observed in female Sprague-Dawley rats administered 0.2 µg/kg 2,3,4,7,8-pentaCDF for 2 years (NTP 2006).

Summary. Studies of the Yusho cohort reported some gastrointestinal alterations. The animal studies indicate that the gastric mucosa is a target of CDFs in monkeys and rats and suggest that guinea pigs and mice are less sensitive rodent species. Only a few studies were performed, however, and congeners other than 2,3,7,8-tetraCDF and 2,3,4,7,8- pentaCDF were not tested.

2.7 HEMATOLOGICAL

Mild normocytic anemia and leukocytosis are fairly consistent findings in Yu-Cheng cohort members (Rogan 1989). In a subsequent study of the Yu-Cheng cohort, an increased prevalence of anemia was observed in women, but not men (Guo et al. 1999). Children living near a municipal waste incinerator in China with increased levels of CDFs also showed higher levels of lymphocytes, mean corpuscular hemoglobin concentration, platelets, mean platelet volume, monocytes, and mean corpuscular hemoglobin when compared to children living outside the area, whereas the levels of hemoglobin, red blood cells, red cell distribution width, platelet distribution width, neutrophils, and hematocrit were lower than the control group (Xu et al. 2019).

2,3,7,8-TetraCDF. Mild anemia, mild lymphopenia, and marked neutrophilia developed in rhesus monkeys following single ≥500 µg/kg dose of 2,3,7,8-tetraCDF (Moore et al. 1979). No hematological alterations were observed in rhesus monkeys exposed to 2.1 or 0.21 µg/kg/day 2,3,7,8-tetraCDF in the diet for 2 or 6 months, respectively (McNulty et al. 1981). In mice administered 300 µg/kg 5 days/week for 30 days, a decrease in total leukocytes was observed; no alterations in total erythrocyte, hemoglobin, or platelet counts were observed (Moore et al. 1979).

No hematological alterations were observed 30 days after guinea pigs were administered a single gavage dose of ≤15 µg/kg 2,3,7,8-tetraCDF (Moore et al. 1979). No alterations in leukocyte counts were observed in guinea pigs administered 1 µg/kg 2,3,7,8-tetraCDF 1 day/week for 6 weeks (Luster et al. 1979a, 1979b).

1,2,3,4,8-PentaCDF. No hematological alterations were observed in rats exposed to 60 or 600 µg/kg/day 1,2,3,4,8-pentaCDF in the diet for 13 weeks (Pluess et al. 1988a).

1,2,3,7,8-PentaCDF. Dietary exposure to ≤20 µg/kg/day for 13 weeks did not result in hematological alterations in rats (Pluess et al. 1988a).

2,3,4,7,8-PentaCDF. In acute-duration studies, rats that were administered single gavage doses of ≥100 µg/kg 2,3,4,78-pentaCDF and evaluated 7–35 days following treatment showed dose-related decreased hemoglobin concentration, mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV) (Brewster et al. 1988). The largest decreases were observed at day 35; the hemoglobin, MCH, and MCV were approximately 6, 9, and 4%, respectively, lower than controls. The toxicological

significance of these small alterations is not known. There were no changes in mean corpuscular hemoglobin concentration (MCHC), red blood cell count, or platelet number, and measurements of white blood cell count were inconclusive. No hematological alterations were observed in rats exposed to ≤20 µg/kg/day for 13 weeks (Pluess et al. 1988b). Single gavage doses of ≤30 µg/kg 2,3,4,7,8-pentaCDF produced no treatment-related hematological changes in Hartley guinea pigs observed for 30 days (Moore et al. 1979).

1,2,3,6,7,8-HexaCDF. No consistent alterations in hematological parameters were observed in rats exposed to 1,2,3,6,7,8-hexaCDF in the diet for 13 weeks (Pluess et al. 1988a).

Mixed Congeners. Dietary exposure to uncharacterized mixtures of two tetra-, four penta-, and four hexaCDFs for 4 weeks caused hemolytic anemia in blood smears, reduced hemoglobin, hematocrit and MCV, and/or increased MCHC in rats at ≥50 µg/kg/day (Oishi and Hiraga 1978; Oishi et al. 1978).

Summary. The results of epidemiological and laboratory animal studies suggest that the hematological system may be a target of toxicity following exposure to high levels of CDFs. No consistent findings were found in studies testing lower dose levels.

2.8 MUSCULOSKELETAL

Several studies have evaluated musculoskeletal endpoints in the Yusho and Yu-Cheng cohorts. A mortality study found an increased prevalence of deaths from diseases of the musculoskeletal system and connective tissue among the Yu-Cheng cohort (Li et al. 2013). Another study of the Yu-Cheng cohort found an increased prevalence of arthritis and herniated discs in men, but not women (Guo et al. 1999). Akahane et al. (2018) observed an increased prevalence of slipped disc, osteoporosis, bony deformity, joint pain, stiff shoulders, and back pain among the Yusho cohort. Studies that measured serum 2,3,4,7,8-pentaCDF levels found an association between serum 2,3,4,7,8-pentaCDF and the prevalence of arthralgia (Kanagawa et al. 2008) and an increased prevalence of osteoporosis among subjects with high serum 2,3,4,7,8-pentaCDF levels (≥72.27 pg/g lipid) (Kondo et al. 2018).

2,3,7,8-TetraCDF. Reduced muscle mass, but no histological alterations in muscle, was observed in guinea pigs administered a single gavage dose of ≥5 µg/kg 2,3,7,8-tetraCDF (Moore et al. 1979). The reduced muscle mass appears to be a manifestation of a generalized wasting syndrome (see Section 2.3).

No histological alterations were observed in muscles or bones of rats administered gavage doses of ≤0.2 µg/kg for 2 years (NTP 2006); this dose level was not associated with alterations in body weight.

2,3,4,7,8-PentaCDF. A single gavage exposure to ≥3 µg/kg 2,3,4,7,8-pentaCDF resulted in a reduction in muscle mass in guinea pigs; no histological alterations were found in muscle (Moore et al. 1979). The reduced muscle mass appears to be a manifestation of a generalized wasting syndrome (see Section 2.3).

Summary. A small number of studies evaluated potential musculoskeletal effects associated with CDF exposure. Studies in the Yusho and Yu-Cheng cohorts provide suggestive evidence, particularly for 2,3,4,7,8-pentaCDF and skeletal effects. No evidence of muscular or skeletal effects were observed in laboratory animals exposed to 2,3,7,8-tetraCDF or 2,3,4,7,8-pentaCDF.

2.9 HEPATIC

Increases in the risk of death from chronic liver disease and/or cirrhosis were observed in several studies of the Yu-Cheng cohort (Li et al. 2013; Tsai et al. 2007; Yu et al. 1997) but were not found in a study of the Yusho cohort (Onozuka et al. 2009; Onozuka et al. 2020). A meta-analysis of the Li et al. (2013) and Onozuka et al. (2009) studies found an increase in deaths from hepatic disease among females (SMR 2.0, 95%CI 1.1–3.6) (Li et al. 2015a).

Mild hepatic alterations were described in the Yusho and Yu-Cheng cohorts (Kuratsune 1989; Rogan 1989). Markedly increased serum triglycerides with unchanged serum cholesterol was an abnormal laboratory finding peculiar to both Yusho and Yu-Cheng cohorts (Okumura et al. 1979; Uzawa et al. 1969). The elevated triglycerides generally persisted for several years following exposure and subsequently declined to normal. Kanagawa et al. (2008) found an association between serum 2,3,4,7,8-pentaCDF levels ≥50 pg/g lipid and increases in total cholesterol levels in the Yusho cohort. In a general population study of 1,374 adults in Japan, an association between serum total CDF TEQ levels in the third quartile (≥4.40–6.60 pg/g lipid) and high triglycerides (≥150 mg/dL) was found; there was no association with low high-density lipoprotein (HDL) levels (<40 mg/dL) for participants with serum total CDF TEQ levels in the fourth quartile (≥6.60 pg/g lipid). A small number of abnormalities in serum levels of liver enzymes or in liver function tests were found in the Yusho cohort (Kuratsune 1989), but elevations in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are fairly consistent findings in the Yu-Cheng cohort (Rogan 1989). Increased urinary excretion of uroporphyrin, but not coproporphyrin or porphobilinogen, is another consistent finding in the Yu-Cheng cohort, including

children born to exposed mothers (Chang et al. 1980; Gladen et al. 1988; Lu et al. 1980). An association between serum total bilirubin levels and serum 2,3,4,7,8-pentaCDF levels ≥50 pg/g lipid has also been found in the Yusho cohort (Kanagawa eta l. 2008).

Ultrastructural changes, particularly alterations in the endoplasmic reticulum, and pleomorphic and enlarged mitochondria, appear to be the predominant morphological finding in Yusho cohort members (Kuratsune 1989). Approximately half of 24 deaths observed in 2,061 Yu-Cheng victims by the end of 1983 were attributed to cirrhosis, unspecified liver diseases with hepatomegaly, or hepatoma (Hsu et al. 1985). Diagnoses were made from clinical symptoms and unspecified laboratory examinations. These findings are inconclusive due to unreported background incidences and high prevalences of hepatitis B, cirrhosis, and liver cancer in Taiwan (Rogan 1989). A follow-up study found an increased prevalence of liver dysfunction among the Yusho cohort (Akahane et al. 2018).

2,3,7,8-TetraCDF. Information on the hepatotoxicity of 2,4,7,8-tetraCDF is limited to three studies in rhesus monkeys. A single dose administration of 1,000 µg/kg resulted in gall bladder and bile duct hypertrophy in monkeys (Moore et al. 1979). Decreases in serum cholesterol were also observed in monkeys administered ≥500 µg/kg; the magnitude of the decrease was not reported. In monkeys exposed to 2.1 µg/kg/day 2,3,7,8-tetraCDF in the diet for 2 months, histological alterations consisting of an increase in the height and number of goblet cells in intrahepatic bile duct epithelium were observed (McNulty et al. 1981). Similar lesions appear to have occurred in monkeys exposed to 0.21 µg/kg/day for 6 months, based on the investigator's statement that the postmortem findings were similar to those reported in a monkey exposed to 2.1 µg/kg/day, although liver lesions were not specifically noted. The liver effects observed in monkeys occurred at lethal doses; interpretation of the results of these studies is limited by the poor quality of the study results reporting.

1,2,3,4,8-PentaCDF. No histological alterations were observed in the liver of rats exposed to 60 or 600 µg/kg/day 1,2,3,4,8-pentaCDF in the diet for 13 weeks (Pluess et al. 1988a).

1,2,3,7,8-PentaCDF. Exposure to 20 µg/kg/day 1,2,3,7,8-pentaCDF in the diet for 13 weeks resulted in increases in liver weight and vacuolization with lipid accumulation and single cell necrosis; no liver lesions were reported at 2 µg/kg/day (Pluess et al. 1988a).

2,3,4,7,8-PentaCDF. Several studies have evaluated the hepatotoxicity of 2,3,4,7,8-pentaCDF following acute, intermediate, or chronic oral exposure. Lipid accumulation in the liver and increases in serum

cholesterol were observed in rats administered a single dose of 100 µg/kg 2,3,4,7,8-pentaCDF (Brewster et al. 1988). No alterations in liver weight (histological examination of the liver not conducted) were observed in rats administered a single dose of 53 µg/kg (Ahlborg et al. 1989). Rats exposed to 0.2 µg/kg/day 2,3,4,7,8-pentaCDF in the diet for 13 weeks exhibited increases in relative liver weight, vacuolization, single cell necrosis, and fatty changes in the liver (Pluess et al. 1988b).

Minimal hepatocellular hypertrophy was observed in rats administered 0.092 µg/kg 5 days/week for 14 weeks (NTP 2006); increases in relative liver weight (10–23%) were also observed at ≥0.006 µg/kg. When the exposure period was extended to 31 weeks, increased incidences of minimal hepatocellular hypertrophy and pigmentation were observed at ≥0.044 µg/kg (NTP 2006); increases in liver weight (14–21%) were observed at ≥0.02 µg/kg. After 2 years of exposure, hepatocellular hypertrophy was observed at ≥0.006 µg/kg (NTP 2006). Other effects observed in the 2-year study include multinucleated hepatocytes, diffuse fatty changes, and pigmentation at ≥0.02 µg/kg; oval cell hyperplasia at ≥0.044 µg/kg; nodular hyperplasia at 0.092 µg/kg; and mild hepatocellular necrosis, minimal to mild bile duct hyperplasia and fibrosis, and mild cholangiofibrosis at 0.2 µg/kg. The effects observed in rats administered 0.2 µg/kg for 2 years were similar to those in rats exposed to 0.2 µg/kg for 30 weeks and allowed to recover for 75 weeks with the exception of the bile duct effects (NTP 2006); the incidences of liver lesions were significantly lower in the stop-exposure group.

1,2,3,6,7,8-HexaCDF. One study evaluated the hepatotoxicity of 1,2,3,6,7,8-hexaCDF. In this study, an increase in relative liver weight and vacuolization with lipid accumulation and single cell necrosis were observed in rats exposed to 2 µg/kg/day in the diet for 13 weeks (Pluess et al. 1988a).

Mixed Congeners. Hepatic effects in rats exposed to an uncharacterized dietary mixture of two tetra-, four penta-, and four hexaCDFs for 4 weeks included increased hepatic uroporphyrin concentrations at 250 µg/kg/day and increased liver weight, lipid content, and serum cholesterol at ≥97 µg/kg/day (Oishi and Hiraga 1978; Oishi et al. 1978).

Mechanisms. CDFs generally induce similar spectra of mild to moderate hepatic effects in animals following single-dose or intermediate-duration oral exposures. Typical changes observed primarily in rats and monkeys included hepatic microsomal enzyme induction, increased serum enzyme levels and liver weight, altered serum cholesterol and triglycerides, fatty and/or necrotic changes in the liver, and bile duct epithelial hyperplasia (Ahlborg et al. 1989; Brewster et al. 1988; Doyle and Fries 1986; Moore et al. 1979; McNulty et al, 1981; Oishi and Hiraga 1978; Oishi et al. 1978; Pluess et al. 1988a, 1988b).

Tetra-, penta-, and hexaCDF congeners substituted in the 2,3,7,8 positions were more hepatotoxic than congeners substituted in other positions. This pattern of toxicity was demonstrated in both acute intraperitoneal studies in rats and guinea pigs and *in vitro* structure-activity relationship studies in rat hepatomas that evaluated induction of hepatic microsomal mixed function oxygenase (MFO) enzymes (e.g., aryl hydrocarbon hydroxylase [AHH], 7-ethoxyresorufin 0-deethylase [EROD]) in rats (Bandiera et al. 1984b; Holcomb et al. 1988; Mason et al. 1985; Safe et al. 1986). Assays for activity of the MFO (AHH) were performed in Sprague-Dawley rats 3 days following a single 40 µg/kg gavage dose of 25 di-, tetra-, penta-, hexa-, hepta-, and octaCDF congeners. Hepatic AHH activity was significantly increased (2.1–4.7-fold) by three congeners with chlorine in all four lateral positions (2,3,7,8-tetraCDF, 2,3,4,7,8-pentaCDF, and octaCDF), and the 2,7- and 2,8-diCDFs, but other doses and endpoints were not evaluated (Doyle and Fries 1986). A single gavage dose of 53 µg/kg 2,3,4,7,8-pentaCDF produced hepatic biochemical changes (increased microsomal EROD activity, decreased vitamin A content) in Sprague-Dawley rats, but there was no change in relative liver weight, and histology was not evaluated (Ahlborg et al. 1989).

As discussed in greater detail in Section 2.20, structure-activity relationships for the induction response are comparable to structure-Ah receptor binding relationships, and the inductive potency *in vitro* correlates well with that observed *in vivo*. These and other findings strongly indicate that induction of certain cytochrome P450IA-dependent microsomal MFO enzymes by CDFs, including AHH and EROD, is mediated by the Ah receptor. Although induction of these enzymes is a characteristic effect of CDFs and related compounds and indicates that interaction with the Ah receptor has occurred, it does not necessarily indicate that hepatotoxic effects will also occur (Poland and Knutson 1982). Based on studies with 2,3,7,8-TCDD and PCBs, there is some evidence that effects of CDFs on lipids (increased serum triglycerides and cholesterol, fatty infiltration of liver) may be Ah receptor-mediated and related to alterations in synthesis of apoproteins involved in lipid formation and utilization (Goldstein and Safe 1989). The extrahepatic biliary epithelial effects may be related to elimination of CDFs and metabolites in the bile (McConnell 1989).

Summary. Epidemiological and laboratory animal studies provide strong evidence that the liver is a target of CDF toxicity. The effects include alterations in serum triglycerides and cholesterol levels, increases in liver weight, lipid accumulation in the liver, and hepatocellular hypertrophy. Bile duct effects have also been reported in laboratory animals. The different congeners appear to have similar liver effects, although there are differences in toxicity and no liver effects were observed in the only non 2,3,7,8-substituted congener that was tested (1,2,3,4,8-pentaCDF). A series of studies conducted by

Pluess et al. (1988a, 1988b) tested the toxicity of several congeners. The LOAELs for lipid accumulation in the liver were 0.2 µg/kg/day for 2,3,4,7,8-pentaCDF, 2 µg/kg/day for 1,2,3,6,7,8-hexaCDF, and 20 µg/kg/day for 1,2,3,4,8-pentaCDF.

2.10 RENAL

Four epidemiological studies evaluated potential renal outcomes. Akahane et al. (2018) reported an increased prevalence of kidney inflammation, hematuria, and proteinuria in the Yusho cohort. No associations were observed between 2,3,4,7,8-pentaCDF or 1,2,3,4,6,7,8-heptaCDF levels and hyperuricemia (defined as serum uric acid levels ≥7 mg/dL for males or ≥6 mg/dL for females) (Zhang et al. 2022). In a study of teens (ages 12–19 years) participating in the National Health and Nutrition Examination Survey (NHANES), no association was found between the incidence of nephropathy and blood 2,3,4,7,8-pentaCDF levels ≥54.02 fg/g whole blood (Everett and Thompson 2016). In a second study using the NHANES data, decreasing trends were observed between kidney function (evaluated using serum creatinine concentrations as a measure of glomerular filtration rate) and 2,3,4,7,8-pentaCDF, 1,2,3,4,7,8-hexaCDF, 1,2,3,6,7,8-hexaCDF, and 1,2,3,4,6,7,8-heptaCDF levels in adults ≥20 years old (Jain 2021).

2,3,7,8-TetraCDF. Acute-duration studies found mild renal effects in animals exposed to lethal doses of CDFs. Hyperplasia of the epithelium in the renal pelvis, ureter, and urinary bladder was observed in guinea pigs up to 30 days after single gavage doses of ≥10 µg/kg 2,3,7,8-tetraCDF (Moore et al. 1979). No histological alterations were observed in the kidneys of mice 30 days after a single, nonlethal gavage dose of 6,000 µg/kg/day 2,3,7,8-tetraCDF (Moore et al. 1979). Blood urea nitrogen (BUN) was increased in rhesus monkeys administered a single gavage dose of ≥1,000 µg/kg 2,3,7,8-tetraCDF only during the period that immediately preceded death, but this was not accompanied by altered kidney weight or histology, and only small numbers were evaluated (Moore et al. 1979).

1,2,3,4,8-PentaCDF. There were no treatment-related kidney histological alterations in rats that ingested ≤600 µg/kg 1,2,3,4,8-pentaCDF via diet for 13 weeks (Pluess et al. 1988a).

1,2,3,7,8-PentaCDF. No histological alterations were observed in the kidneys of rats exposed to ≤20 µg/kg 1,2,3,7,8-pentaCDF in the diet for 13 weeks (Pluess et al. 1988a).

2,3,4,7,8-PentaCDF. Increased relative kidney weight, decreased absolute kidney weight, and 64% increased BUN were found in rats observed for 35 days following single gavage doses of ≥500, ≥1,000, and 2,000 µg/kg 2,3,4,7,8-pentaCDF, respectively (Brewster et al. 1988). Reduced body weight contributed to the increased relative kidney weights. There were no histological alterations in the kidneys or bladder in any of the treated rats. Because both organ weight and functional (BUN) changes occurred at 2,000 mg/kg, this dose is a LOAEL.

In an intermediate-duration study, no histological alterations were observed in rats exposed to ≥20 µg/kg/day 2,3,4,7,8-pentaCDF in the diet for 13 weeks (Pluess et al. 1988b). Chronic gavage exposure to 2,3,4,7,8-pentaCDF resulted in an increased incidence of nephropathy in female rats administered ≥0.044 µg/kg 5 days/week for 2 years (NTP 2006).

1,2,3,6,7,8-HexaCDF. Dietary exposure to ≤20 µg/kg/day 1,2,3,6,7,8-hexaCDF did not result in alterations in kidney histology in rats exposed for 13 weeks (Pluess et al. 1988a).

Mixed Congeners. In intermediate-duration studies, kidney histology was not evaluated in Sprague-Dawley rats exposed to ≤960 µg/kg/day of an uncharacterized dietary mixture of two tetra-, four penta-, and four hexaCDFs for 4 weeks (Oishi et al. 1978). However, based on unchanged relative kidney weight, no adverse effects were observed.

Summary. The kidney does not appear to be a sensitive target of CDF toxicity following acute or intermediate exposure. Mild to moderate renal effects have been observed in guinea pigs, rats, and monkeys exposed to lethal doses of 2,3,7,8-tetraCDF or 2,3,4,7,8-pentaCDF. Chronic nonlethal doses of 2,3,4,7,8-pentaCDF did result in nephropathy in rats.

2.11 DERMAL

Effects in the skin and eyes are the most obvious manifestations of exposure in the Yusho and Yu-Cheng cohorts; they were evaluated in a number of studies and were observed in the majority of cases (Hsu et al. 1993; Kuratsune 1989; Lu and Wu 1985; Rogan 1989). Characteristic skin changes included marked enlargement, elevation and keratotic plugging of follicular orifices, comedones (blackhead) formation, acneiform eruptions, hyperpigmentation, hyperkeratosis, and deformed nails. The acne most commonly developed in the face and other parts of the head, axillae, trunk, and external genitalia, with follicular plugging occurring in the axillae, groin, glenoid regions such as elbow and knee flexures, trunk, thigh,

and outer aspect of the forearm. Dark-colored pigmentation frequently occurred in the gingival and buccal mucosa, lips, and nails and improved only gradually in most patients. Decades after the Yusho and Yu-Cheng incidents, increases in the prevalence of acne, black comedones, and abnormal nails are still reported (Akahane et al. 2018; Guo et al. 1999; Mitoma et al. 2015). Associations between serum 2,3,4,7,8-pentaCDF and the prevalence of the dermal effects have also been reported (Imamura et al. 2007; Kanagawa et al. 2008; Mitoma et al. 2015); the results of these three studies are summarized in Table 2-6.

Table 2-6. Results of Epidemiological Studies Evaluating Exposure to CDFs and Dermal Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Imamura et al. 2007 Retrospective, 241 adults in Yusho cohort (Japan)	Serum total CDF mean of 264.26 pg/g lipid	Recent purulent skin eruptions	↑
		Recurrence of cystic lesions	↑
		History of acneiform eruptions	↑
		History of pigmentation	↑
		Black comedones	↑
		Acneiform eruptions	↑
		Scar formation	↑
		Nail deformity	↑
Kanagawa et al. 2008 Retrospective, 501 adults in Yusho cohort (Japan)	Serum 2,3,4,7,8-pentaCDF ≥50 pg/g lipid	Acneiform eruptions	↑
Mitoma et al. 2015 Retrospective, 352 adults in Yusho cohort (Japan)	Serum 2,3,4,7,8-pentaCDF mean 130.8 pg/g lipid	Severity of black comedones and scar formation	↑
		Scar formation	↑

↑ = association between biomarker level and outcome; ↓ = inverse association between biomarker level and outcome; ↔ = no association between biomarker level and outcome; CDF = chlorodibenzofuran

2,3,7,8-TetraCDF. Rhesus monkeys that were treated with single, nonlethal (500 µg/kg) or lethal (≥1,000 µg/kg) doses of 2,3,7,8-tetraCDF and observed for 60 days developed progressive and dose-related skin lesions (Moore et al. 1979). These included dry leathery skin, facial edema, loss of fingernails, exudate with occlusion and squamous metaplasia of ear canal (ceruminous) glands, and epidermal hyperkeratosis; dilation of sebaceous gland ducts and follicular hyperkeratosis were also observed at 1,500 µg/kg. No skin histological alterations were observed in guinea pigs 30 days after

single gavage doses of ≤15 µg/kg 2,3,7,8-tetraCDF or in C57BU6Fh mice administered a single dose of 6,000 µg/kg 2,3,7,8-tetraCDF (Moore et al. 1979).

Dermal lesions also developed in rhesus monkeys treated with 2,3,7,8-tetraCDF in intermediate-duration studies (McNulty et al. 1981). Dietary dosages of 0.21 µg/kg/day for ≤6 months caused partial atrophy of sebaceous glands and hyperkeratotic nail beds. Similar exposure to a higher dosage of 2.1 µg/kg/day caused more severe skin changes, including thickening and partial facial hair loss after 1 month, body hair and nail loss, and absent sebaceous glands. Surviving monkeys were completely recovered 2–3 months after either exposure.

2,3,4,7,8-PentaCDF. Nail hemorrhages were observed in rats administered a single dose of 500 µg/kg 2,3,4,7,8-pentaCDF (Brewster et al. 1988). No dermal alterations were observed in guinea pigs receiving a single dose of 30 µg/kg (Moore et al. 1979) or in rats administered via gavage ≤0.2 µg/kg 2,3,4,7,8-pentaCDF 5 days/week for 2 years (NTP 2006).

Mixed Congeners. Chloracne-like lesions developed on the ears of rats exposed to 960 µg/kg/day dietary dosages of an uncharacterized mixture of two tetra-, four penta-, and four hexaCDFs for 4 weeks (Oishi et al. 1978).

Summary. Effects in the skin are the most obvious manifestations of CDF toxicity in humans and animals. The studies in animals, although limited by number of congeners and species tested, indicate that high doses of 2,3,7,8-tetraCDF and 2,3,4,7,8-pentaCDF cause dermal alterations and that monkeys are more sensitive than rodents.

2.12 OCULAR

Most subjects showed eye discharge and other severe ocular effects during the acute phase of the Yusho and Yu-Cheng syndrome (Fu 1984; Kuratsune 1989; Lu and Wu 1985; Rogan 1989). These effects include meibomian gland changes (enlargement, inflammation, hypersecretion of cheese-like material) and dark-colored pigmentation of the conjunctivae and eyelids. Post-exposure, improvement of the ocular changes was gradual and occurred with improvement of dermal effects. Increased prevalences of excessive eye discharge, cataracts, near sightedness, and “lazy eye” were observed in the Yusho cohort several decades after the rice oil exposure incident (Akahane et al. 2018).

2,3,7,8-TetraCDF. Ocular effects observed in monkeys administered a single dose of ≥500 µg/kg 2,3,4,7,8-pentaCDF included loss of eyelashes, exudate with occlusion, and squamous metaplasia of eyelid (meibomian) glands (Moore et al. 1979). No eye histological alterations were observed in guinea pigs 30 days after single gavage doses of ≤15 µg/kg 2,3,7,8-tetraCDF (Moore et al. 1979).

Ocular lesions also developed in rhesus monkeys treated with 2,3,7,8-tetraCDF in intermediate-duration studies (McNulty et al. 1981). Periorbital edema and meibomian gland enlargement were observed at 0.21 µg/kg/day. Similar exposure to a higher dosage of 2.1 µg/kg/day resulted in eyelid reddening after 1 month. Surviving monkeys completely recovered 2–3 months after exposure termination.

2,3,4,7,8-PentaCDF. No ocular effects were observed in guinea pigs administered a single dose of ≤30 µg/kg 2,3,4,7,8-pentaCDF (Moore et al. 1979) or in rats administered gavage doses of ≤0.2 µg/kg 2,3,4,7,8-pentaCDF 5 days/week for 2 years (NTP 2006).

Summary. Ocular effects have been observed in humans and monkeys exposed to high doses of CDFs, but have not been observed at lower doses in rodent studies.

2.13 ENDOCRINE

Endocrinological evaluations of the Yu-Cheng cohort found a tendency for increased urinary excretion of 17-ketosteroids and 17-hydroxycorticosteroids (Nagai et al. 1971). Effects on reproductive endocrinology in the Yu-Cheng and Yusho cohorts have also been reported (see Section 2.16). Three studies evaluated potential thyroid effects. Increased prevalences of hypothyroidism in the Yusho cohort (Akahane et al. 2018) and goiter in the Yu-Cheng cohort (Guo et al. 1999) have been reported. Another study reported that serum triiodothyronine (T3), T4, free T4, and thyroid stimulating hormone (TSH) levels were within the normal range in the Yusho cohort (Nagayama et al. 2001). In a study examining 10-year-old children living near a municipal waste incinerator in China, higher levels of multiple CDFs and free T3 were observed compared to controls; no differences were observed in the levels of free T4, T3, T4, or TSH (Xu et al. 2019).

Several epidemiological studies have evaluated possible associations between diabetes and CDF exposure and found conflicting results. An increase in the prevalence of diabetes was reported in the Yusho cohort (Akahane et al. 2018) and among women in the Yu-Cheng cohort, particularly in women with a history of chloracne (Wang et al. 2008). However, another study did not find an increase in the risk of deaths from

diabetes mellitus among the Yu-Cheng cohort (Tsai et al. 2007). Similarly, no increase in deaths from diabetes mellitus were found in the men and women of the Yusho cohort examined through 2007 (Onozuka et al. 2009) and 2017 (Onozuka et al. 2020). Uemura et al. (2009) reported an association between serum total CDF TEQ levels in the third quartile (≥4.40–<6.60 pg/g lipid) and HbA1c levels of >5.6% or physician-diagnosed diabetes among Yusho cohort participants. A study of NHANES participants with blood 2,3,4,7,8-pentaCDF levels ≥51.74 fg/g whole blood found an increased risk of diabetes with or without nephropathy (Everett and Thompson 2014).

2,3,7,8-TetraCDF. Several studies have evaluated potential endocrine effects in laboratory animals orally exposed to 2,3,7,8-tetraCDF and reported thyroid and adrenal effects. Decreases in serum total T4 levels were reported in rats following exposure to 2,3,7,8-tetraCDF for 4 days. The magnitude of the decrease was approximately 26% in rats exposed to 1 µg/kg/day (Ross et al. 2000), 30% at 4.65 µg/kg/day (Crofton et al. 2005), and 50% at 10 µg/kg/day (Ross et al. 2000). Administration of a single dose of 2,3,7,8-tetraCDF did not result in histological alterations in the thyroid of monkeys administered ≤1,500 µg/kg, mice administered 6,000 µg/kg, or guinea pigs administered 15 µg/kg (Moore et al. 1976, 1979); it is noted that the animals were allowed to recover for 30 days (mice and guinea pigs) or 60 days (monkeys) prior to sacrifice.

Adrenal hemorrhage was found in Hartley guinea pigs that received single, lethal, gavage doses of ≥10 µg/kg 2,3,7,8-tetraCDF (Moore et al. 1979). No adrenal effects were observed in monkeys (≤1,500 µg/kg) or mice (≤6,000 µg/kg) (Moore et al. 1976, 1979). No consistent effects on serum hydrocortisone levels occurred in Hartley guinea pigs treated by gavage with weekly ≤1 µg/kg/day doses of 2,3,7,8-tetraCDF for 6 weeks (Luster et al. 1979a, 1979b). The Moore et al. (1976, 1979) study did not find histological alterations in the pancreases of the three tested species.

1,2,3,4,8-PentaCDF. One study evaluated the potential endocrine toxicity of oral exposure to 1,2,3,4,8-pentaCDF. No histological alterations were observed in the adrenal or thyroid/parathyroid glands of rats exposed to 600 µg/kg/day in the diet for 13 weeks (Pluess et al. 1988a).

1,2,3,7,8-PentaCDF. Information on the toxicity of 1,2,3,7,8-pentaCDF to the endocrine system is limited to two 4-day studies that measured serum total T4 levels. The magnitude of the decrease was 15% at 10 µg/kg/day (Ross et al. 2000), 30% at 15.6 µg/kg/day (Crofton et al. 2005), and 40% at 30 µg/kg/day (Ross et al. 2000).

In an intermediate-duration study, no histological alterations were observed in the adrenal or thyroid glands of rats exposed to ≤20 µg/kg/day 1,2,3,7,8-pentaCDF in the diet for 13 weeks (Pluess et al. 1988a).

2,3,4,7,8-PentaCDF. Similar to 2,3,7,8-tetraCDF and 1,2,3,7,8-pentaCDF, a 4-day exposure to 2,3,4,7,8-pentaCDF resulted in decreases in serum total T4 levels. A 27–30% decrease was observed at 27.5–30 µg/kg/day (Crofton et al. 2005; Ross et al. 2000) and a 47% decrease was observed at 90 µg/kg/day (Ross et al. 2000). Administration of 2,3,4,7,8-pentaCDF to female rats 5 days/week for 14 weeks resulted in thyroid gland follicular cell hypertrophy at ≥0.044 µg/kg and a decrease in serum total T4 levels (25%) at ≥0.092 µg/kg (NTP 2006). Decreases in serum total T4 levels (16%) were observed in rats following gavage administration of ≥0.006 µg/kg 2,3,4,7,8-pentaCDF 5 days/week for 31 weeks; increased total T3 levels were observed at ≥0.092 µg/kg (NTP 2006). No histological alterations were observed in the thyroid gland after 31 weeks of exposure to ≤0.2 µg/kg (NTP 2006). Chronic administration of 2,3,4,7,8-pentaCDF resulted in follicular cell hypertrophy in the thyroid gland at ≥0.02 µg/kg (NTP 2006). After 53 weeks of exposure, alterations in thyroid hormone levels included decreases (22%) in serum total T4 levels at ≥0.044 µg/kg and increases (23%) in serum total T3 levels at ≥0.092 µg/kg; there were no alterations in TSH levels (NTP 2006).

Administration of a single dose of 10 µg/kg 2,3,4,7,8-pentaCDF resulted in adrenal hemorrhage in guinea pigs (Moore et al. 1979). No alterations in adrenal histology were observed in rats administered dietary dosages ≤20 µg/kg/day 2,3,4,7,8-pentaCDF for 13 weeks (Pluess et al. 1988b). NTP (2006) also reported cystic degeneration in the adrenal cortex at ≥0.006 µg/kg and arterial chronic inflammation in the pancreas at 0.2 µg/kg in the chronic-duration study.

1,2,3,6,7,8-HexaCDF. Adrenal and thyroid/parathyroid histology was normal in rats administered dietary dosages of ≤20 µg/kg/day 1,2,3,6,7,8-hexaCDFfor 13 weeks (Pluess et al. 1988a, 1988b). These dosages were sublethal except for 10 µg/kg/day 2,3,4,7,8-pentaCDF.

OctaCDF. In the only study evaluating potential thyroid effects, Crofton et al. (2005) did not find any significant alterations in the serum total T4 levels in rats administered octaCDF for 4 days. The investigators suggested that the lack of an effect may be due to the poor absorption of octaCDFs.

Mechanisms. The mechanisms by which CDFs decrease serum T4 levels have not been fully elucidated. A likely mechanism involves the catabolism of T4 via T4 glucuronidation. CDFs induce hepatic uridine 5′-diphospho-glucuronosyltransferase (UDPGT) likely through an Ah receptor-mediated pathway (Ross

et al. 2000). Exposure of rats to 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, or 2,3,4,7,8-pentaCDF resulted in decreases in serum T4 levels and increases in UDPGT activity (Ross et al. 2000). However, the magnitude of the decrease in T4 was not directly related to the increase in UDPGT activity. For example, there was a 50% decrease in serum T4 levels in rats exposed to 100 µg/kg/day and an 11 % increase in UDGPT activity at this dose level.

Microsomal enzyme inducers can induce UDPGT and increase the biliary excretion of T4, resulting in a reduction of serum T4 levels. Compensatory increases in serum TSH levels have been observed for some chemicals such as phenobarbital and pregnenolone-16α-carbonitrile (PCN). However, other chemicals such as PCB (Aroclor 1254) and 3-methylcholanthrene do not increase serum TSH levels (Hood and Klaassen 2000; Hood et al. 2003; Richardson and Klaassen 2010). Although most of the research was conducted in rats, decreases in serum T4 have also been observed in mice exposed to 3-methyl-cholanthrene and PCB (Hood et al. 2003). However, slight increases in TSH levels were also observed in mice. CDFs appear to work by a similar mechanism as methylcholanthrene and PCB. Decreases in serum T4 levels were reported in rats exposed to 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, and 1,2,3,6,7,8-hexaCDF (Crofton et al. 2005; NTP 2006; Ross et al. 2000); no changes in TSH levels were observed in rats exposed to 2,3,4,7,8-pentaCDF (NTP 2006). Epidemiological studies on the potential thyroid toxicity of CDFs are inconclusive. Occupational exposure studies involving CDDs (ATSDR 1998, 2012) and PCBs (ATSDR 2000) have found alterations in thyroid hormone levels.

Summary. The data from the available epidemiological studies on the association between CDF exposure and thyroid effects are inconclusive. Animal studies provide strong evidence that oral exposure to 2,3,7,8-substituted congeners result in decreases in serum T4 levels. The results of studies evaluating 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, and 2,3,4,7,8-pentaCDF found greater decreases in serum T4 levels in rats exposed to 2,3,7,8-tetraCDF compared to the two pentaCDF congeners (Crofton et al. 2005; Ross et al. 2000). In addition to the thyroid effects, a chronic oral study in rats found histological alterations in the adrenals (NTP 2006).

2.14 IMMUNOLOGICAL

Clinical observations strongly suggest that the Yusho and Yu-Cheng cohorts experienced frequent or more severe skin and respiratory infections and lowered resistance to illness (Kuratsune 1989; Rogan 1989). Various changes in immune status were reported among the Yusho and Yu-Cheng cohorts,

including decreased serum IgA and IgM levels and lymphocyte subpopulations, diminished phagocyte complement and IgG receptors, and diminished delayed-type skin hypersensitivity response (Chang et al. 1981, 1982a, 1982b; Lu and Wu 1985; Nakanishi et al. 1985; Shigematsu et al. 1971). Higher levels of certain interleukins suggestive of dysregulated T_H17 cell-mediated immune responses were also observed (Kuwatsuka et al. 2014). Kamio et al. (2020) found that 31 Yusho patients had a higher proportion of natural killer cells (but not T cells), increased CTLA-4 (marker of T cell activation), and decreased IFN-γ and IL-36γ (markers of natural killer cell activation) compared to matched controls. Children living near a municipal waste incinerator in China with increased levels of CDFs also showed a higher percentage of B cells than children living outside the area, although no differences were observed in the percentages of T lymphocytes, cytotoxic T lymphocytes, helper T lymphocytes, or natural killer lymphocytes (Xu et al. 2019).

Follow-up studies of the Yusho and Yu-Cheng cohorts have found alterations in the prevalence of immune-related diseases such as asthma, rheumatoid arthritis, and drug and skin allergies (Akahane et al. 2018; Guo et al. 1999). Tsai et al. (2007) found an increase in deaths from systemic lupus erythematosus in women in the Yu-Cheng cohort; most of the deaths occurred in the 1988–1995 time period rather than the 1996–2003 time period.

Decreased thymus weight and thymic atrophy, characterized by lymphoid cell loss, involutions, and/or lack of corticomedullary differentiation, have been consistently observed in animals exposed to CDFs. Thymus weight decreases were often pronounced, particularly at lethal doses where reductions as high as 80–90% were observed.

2,3,7,8-TetraCDF. Acute- and intermediate-duration studies of 2,3,7,8-tetraCDF provide strong evidence that the immune system, particularly the thymus, is a target of CDF toxicity. In monkeys, thymic atrophy was reported following administration of a lethal dose of 1,000 µg/kg 2,3,7,8-tetraCDF (Moore et al. 1979) and dietary exposure to 2.1 or 0.21 µg/kg/day for 2 or 6 months (McNulty et al. 1981). A marked reduction of thymus size was also observed in guinea pigs administered a single dose of 5 µg/kg (Moore et al. 1979) and in mice administered 300 µg/kg 5 days/week for 30 days (Moore et al. 1979). Other histological alterations in the immune system include loss of lymphoid cells in the thymic cortex, hypocellularity of bone marrow, and "lymphoid elements in the spleen and Peyer's patches" were observed in guinea pigs administered a single dose of 10 µg/kg (no additional information was provided) (Moore et al. 1979).

One study evaluated immune function in guinea pigs administered 2,3,7,8-tetraCDF 1 day/week for 6 weeks (Luster et al. 1979a, 1979b). No alterations in humoral immunity were found as evaluated by measuring serum proteins (albumin, α-globulins, β-globulins, γ-globulins, and IgG levels) or the response to immunization with bovine gamma globulin; however, a decreased lymphoproliferative response to lipopolysaccharide provides an indication that humoral immunity was compromised. Cell-mediated immunity was impaired in the guinea pigs administered 0.5 µg/kg as evidenced by the impaired lymphoproliferative response to phytohemagglutinin in the 0.5 µg/kg group; there was no effect on the response to concanavalin A.

1,2,3,4,8-PentaCDF. No alterations in thymus or spleen weight or histology were observed in rats exposed to 600 µg/kg/day 1,2,3,4,8-pentaCDF in the diet for 13 weeks (Pluess et al. 1988a).

1,2,3,7,8-PentaCDF. A 13-week exposure of rats to 20 µg/kg/day 1,2,3,7,8-pentaCDF resulted in decreases in thymus weight and minimal thymic atrophy (Pluess et al. 1988a). No alterations were observed in the spleen.

2,3,4,7,8-PentaCDF. Decreases in thymus weight have been observed in rats and guinea pigs administered single doses of 2,3,4,7,8-pentaCDF. Taura et al. (2014) estimated an ED_{50} (50% decrease in thymus weight) of 71.9 µg/kg in pubertal rats (5 weeks of age) receiving a single gavage dose of 2,3,4,7,8-pentaCDF. A 100 µg/kg dose resulted in 30–90% decreases in thymic weight in rats (Brewster et al. 1988). At 500 µg/kg, thymic atrophy was observed. No alterations in thymus weight were observed in rats administered 53 µg/kg (Ahlborg et al. 1989). Guinea pigs appear to be more sensitive than rats; a reduction in thymus size was observed at 3 µg/kg and a loss of lymphoid cells were observed in the thymic cortex at 10 µg/kg.

Intermediate-duration exposure resulted in decreases in thymus weight at 0.2 µg/kg and thymic atrophy at 2 µg/kg in rats exposed for 13 weeks (Pluess et al. 1988b) and thymic atrophy in rats administered 0.2 µg/kg 5 days/week for 31 weeks (NTP 2006). No alterations in thymus weight or pathology were observed in rats administered 0.2 µg/kg 5 days/week for 14 weeks (NTP 2006). In a chronic study, an increase in the severity of thymic atrophy was observed in rats administered 0.2 µg/kg 5 days/week for 2 years (NTP 2006).

Laboratory animal studies have also reported histological alterations in other tissues. Lymphoid depletion was observed in the spleen of rats administered a single dose 500 µg/kg (Brewster et al. 1988). In guinea

pigs administered a 10 μg/kg dose, loss of lymphoid cells in the thymic cortex, hypocellularity of bone marrow, and "lymphoid elements in the spleen and Peyer's patches were observed" (no additional information was provided) (Moore et al. 1979).

One study evaluated immune function following a single dose exposure to 2,3,4,7,8-pentaCDF (Johnson et al. 2000). An ED_{50} of 10.119 was calculated for a 50% reduction in the response to sheep red blood cells (SRBCs).

1,2,3,6,7,8-HexaCDF. Severe thymic atrophy was observed in rats exposed to 20 μg/kg/day 1,2,3,6,7,8-hexaCDF in the diet for 13 weeks; no alterations were observed at 2 μg/kg/day (Pluess et al. 1988a).

1,2,3,4,6,7,8-HeptaCDF. An ED_{50} of 208 μg/kg for decreased antibody response to SRBCs was estimated in mice receiving a single dose of 1,2,3,4,6,7,8-heptaCDF (Kerkvliet et al. 1985).

Mixed Congeners. A decrease in thymus weight was observed in rats fed ≈44 μg/kg/day of a CDF mixture similar to that in Yusho oil for 10 days (Kunita et al. 1984). Dietary exposure to an uncharacterized mixture of two tetra-, four penta-, and four hexaCDFs for 4 weeks caused decreased thymus weight at ≥97 μg/kg/day in Sprague-Dawley rats (Oishi et al. 1978). Thymus weights were decreased in ICR/JCL mice treated with four weekly 100 μg/kg gavage doses of a mixture containing 88% pentaCDFs and 12% tetraCDFs (congeners not identified) (Oishi and Hiraga 1980); spleen weights were unaffected.

Studies of mortality from injected Escherichia coli lipopolysaccharide endotoxin in mice treated with four weekly 100 μg/kg doses of a pentaCDFs/tetraCDFs mixture were inconclusive (Oishi and Hiraga 1980).

Mechanisms. The immunotoxicity of CDFs, chlorinated dibenzo-*p*-dioxins (CDDs), and PCBs appears to be associated with binding to the Ah receptor (Section 2.3.5) (Harper et al. 1993; Vos and Luster 1989). This receptor has been identified in various tissues, including human and murine lymphocytes, thymic epithelial cells, and bone marrow cells. Thymic atrophy and suppressed antibody responses, induced by CDF, 2,3,7,8-TCDD, and/or PCB congeners, have been shown to be Ah receptor-mediated. Although there is evidence that the immunotoxicity of CDFs and related chlorinated aromatic compounds is associated with the Ah receptor, the mechanisms responsible for toxicity following interaction of the receptor-ligand complex with the Ah locus are unknown (Vos and Luster 1989). There is some evidence

that additional loci may be involved and that these compounds can directly affect the thymic epithelium, leading to thymic atrophy and suppression of cell-mediated immunity.

Studies with 2,3,7,8-TCDD, as reviewed by Marshall and Kerkvliet (2010), provide information on mechanisms of immunotoxicity that are likely relevant to CDFs. The major cells of the immune system express Ah receptor; the effects associated with 2,3,7,8-TCDD Ah receptor activation is dependent on the cell type, the cell's activation status, and the type of antigenic stimulation. The innate and adaptive immune systems are affected. In the innate system, 2,3,7,8-TCDD alters the function of the dendritic cells. Initially, the dendritic cells expressed increased levels of major histocompatibility complex (MHC) class II molecules, intercellular adhesion molecule type 1 (ICAM-1) and CD24 adhesion molecules, costimulatory molecule CD40, and IL-2; an increase in T-cell stimulating ability was also observed. However, 4–7 days after mice were exposed to 2,3,7,8-TCDD, there were decreases in the number of dendritic cells in the spleen and increased Fas-mediated apoptosis of bone marrow derived dendritic cells. The decrease in the number of dendritic cells likely results in a decrease in the strength and duration of a T-cell-mediated response. Other Ah receptor-mediated effects on dendritic cells included altered signaling of the canonical and noncanonical NF-κB pathways. In the adaptive immune system, 2,3,7,8-TCDD has a number of targets including B cells, $CD4^+$ T cells, and $CD8^+$ T cells. 2,3,7,8-TCDD exposure can result in the premature cessation of T-cell proliferation and inhibition of cytotoxic T lymphocyte activation. 2,3,7,8-TCDD inhibits $CD4^+$ T cell differentiation into T helper 1, 2, and 17 cells (Th1, Th2, and Th17) and suppresses Th1-, Th2-, and Th17-mediated responses. 2,3,7,8-TCDD also appears to induce the development of adaptive T regulatory cells (Tregs) that do not express Foxp3 and increase the number of Foxp3 positive Tregs.

A study with 2,3,7,8-TCDD in mice found alterations in the gut microbiome, which resulted in increased production of myeloid derived-suppressor cells (MDSCs) via Ah receptor activation (Neamah et al. 2020). The MDSCs have been shown to be immunosuppressive.

Summary. In conclusion, available studies suggest that CDFs have the potential to impair immunocompetence and that thymic effects are part of the spectrum of adverse effects on the immune system. Immunologic effects were observed in all animal species tested, but mice appear to be less sensitive than other rodents and monkeys. Based on the animal data, the most potent congeners are those substituted in the 2,3,7,8- positions, particularly, 2,3,4,7,8-pentaCDF.

2.15 NEUROLOGICAL

Various neurological symptoms, including numbness, weakness and neuralgia of limbs, hypesthesia, and headaches, were common in Yusho and Yu-Cheng victims (Chia and Chu 1984, 1985; Kuratsune 1989; Rogan 1989). Conduction velocities were reduced in sensory nerves (radial and/or sural) in 9 of 23 Yusho cohort members examined soon after poisoning (Kuroiwa et al. 1969). Sensory fibers appear to be preferentially affected, as conduction velocities in motor nerves (ulnar and tibial) were reduced in only two cases and motor functions were normal. Follow-up studies were not performed on the Yusho cohort, but disappearance of related symptoms and signs indicated that the effects on nerve conduction did not persist. Reduced sensory and motor nerve conduction velocities also occurred in Yu-Cheng cohort members (Chen et al. 1985b; Chia and Chu 1984, 1985). Evaluation of 110 members in the Yu-Cheng cohort within 1 year of exposure showed abnormally slow sensory nerve (median and ulnar) and motor nerve (tibial and peroneal) conduction velocities in ≈44 and 22% of the patients, respectively (Chen et al. 1985b). All of the subjects had developed eye and skin manifestations of toxicity, but there were no significant correlations between nerve conduction values and blood levels of PCBs, CDFs or PCQs. Electroencephalographic examination of Yu-Cheng cohort members did not show any abnormalities potentially indicative of central nervous system damage (Chia and Chu 1984, 1985). An additional study examined physical functions including functional reach, gait speed, and hand/toe grip strength, functions that may be related to frailty, osteoporosis, and the risk of falling, in Yusho patients (65 men, mean age 65.7 years; and 77 women, mean age 64.7 years). Associations were observed between total CDF TEQ and functional reach and hand grip strength in men, while similar trends were observed in women (Fukushi et al. 2019).

Several studies have followed up on the Yusho and Yu-Cheng cohorts. Yu et al. (1997) did not find increases in deaths from mental or psychoneurotic disorders among the Yu-Cheng cohort during the 1979–1991 time period. Members of the Yusho cohort reported a number of neurological effects including increased prevalence of headaches, nerve pain, forgetfulness, prone to losing temper and irritability, insomnia, anxiety, vertigo, hearing loss, numbness in extremities, body cramping, and muscle pain (Akahane et al. 2018). An increased prevalence of headaches was also reported in the Yu-Cheng cohort (Guo et al. 1999). Associations between serum total CDF levels (mean of 264.26 pg/g lipid) and the prevalence of numbness (Imamura et al. 2007) was found in the Yusho cohort. In another study of the Yusho cohort, depression and severe insomnia were reported in participants with serum 2,3,4,7,8-pentaCDF levels of ≥72.27 pg/g lipid (Kondo et al. 2018). A study of elderly cohort Yu-Cheng cohort members reported a reduced learning ability among females, with no effects in males (Lin et al.

2008, 2010). Neurobehavioral deficits have also been observed in children born to mothers in the Yu-Cheng cohort (see Section 2.17).

2,3,7,8-TetraCDF. Single gavage doses of ≤15 µg/kg to guinea pigs or ≤6,000 µg/kg to mice produced no histological alterations in the brain in the animals examined 30 days after exposure (Moore et al. 1976, 1979).

2,3,4,7,8-PentaCDF. Signs of toxicity in rats given single, lethal doses of 2,3,4,7,8-pentaCDF included piloerection, splayed and hunched posture, and hypoactivity at ≥1,000 µg/kg, and tremors and lacrimation in one animal at 2,000 µg/kg (Brewster et al. 1988). No histological alterations were observed in the brain of guinea pigs administered a single dose of ≤30 µg/kg 2,3,4,7,8-pentaCDF and examined 30 days later (Moore et al. 1979) or in rats administered via gavage ≤0.2 µg/kg 5 days/week for 2 years (NTP 2006).

Mixed Congeners. Dietary exposure to an uncharacterized mixture of two tetra-, four penta-, and four hexaCDFs for 4 weeks caused grossly observable cerebral edema and "flabby" brain appearance in Sprague-Dawley rats at ≥97 µg/kg/day, but slight fluid accumulation also occurred in the thorax and abdomen (Oishi et al. 1978).

Summary. Studies of the Yusho and Yu-Cheng cohorts have consistently reported neurological effects. Studies in laboratory animals have not reported direct effects on the central nervous system; observed effects were probably secondary to other changes (e.g., wasting syndrome, stress) occurring in intoxicated or dying animals. However, there were no studies evaluating potential effects on neurobehavioral function.

2.16 REPRODUCTIVE

The potential reproductive toxicity of CDFs in humans was evaluated in studies of the Yusho and Yu-Cheng cohorts, in populations living in areas of Vietnam that were highly contaminated with Agent Orange, and in the general population. Studies that evaluated possible associations between serum levels of CDFs and a health outcome are summarized in Table 2-7 (Cai et al. 2011; Martinez-Zamora et al. 2015; Sun et al. 2016, 2017; Tsukimori et al. 2008; Van Luong et al. 2018).

Table 2-7. Results of Epidemiological Studies Evaluating Exposure to CDFs and Reproductive Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Cai et al. 2011 Case control, 10 cases with endometriosis, 7 controls (Japan)	Serum total CDF 7.5 pg/g lipid (cases) and 6.9 pg/g lipid (controls)	Endometriosis	↔
Martinez-Zamora et al. 2015 Case control, 30 cases with deep infiltrating endometriosis and 30 controls (Spain)	Serum mean 2,3,4,7,8-pentaCDF 4.98 pg/g lipid (cases) and 3.95 pg/g lipid (controls)	Deep infiltrating endometriosis	↑
Shi et al. 2020 Cross-sectional, 74 men living near an electronic waste area (China)	Serum CDFs-TEQ low group 3.80–6.31 pg/g lipid; moderate group, 6.32–11.33 pg/g lipid; high group, ≥11.34 pg/g lipid (reference group <3.80 pg/g lipid)	DHT	↔
		Testosterone	↔
		DHEA	↑ Low group only
		A-dione	↑ High group only
		3β-HSD	↔
Sun et al. 2016 Cross-sectional, 97 men living in areas highly exposed to Agent Orange and 85 controls (Vietnam)	Serum geometric mean 2,3,7,8-tetraCDF 0.16 pg/g lipid (exposed) and 0.15 pg/g lipid (controls)	Prostate specific antigen	↔
	Serum geometric mean 1,2,3,7,8-pentaCDF 0.06 pg/g lipid (exposed) and 0.04 pg/g lipid (controls)	Prostate specific antigen	↔
	Serum geometric mean 2,3,4,7,8-pentaCDF 3.98 pg/g lipid (exposed) and 2.14 pg/g lipid (controls)	Prostate specific antigen	↔
	Serum geometric mean 1,2,3,4,7,8-hexaCDF 2.75 pg/g lipid (exposed) and 0.44 pg/g lipid (controls)	Prostate specific antigen	↔
	Serum geometric mean 1,2,3,6,7,8-hexaCDF 2.09 pg/g lipid (exposed) and 0.49 pg/g lipid (controls)	Prostate specific antigen	↔
	Serum geometric mean 1,2,3,7,8,9-hexaCDF 0.31 pg/g lipid (exposed) and 0.26 pg/g lipid (controls)	Prostate specific antigen	↔

Table 2-7. Results of Epidemiological Studies Evaluating Exposure to CDFs and Reproductive Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Serum geometric mean 2,3,4,6,7,8-hexaCDF 0.38 pg/g lipid (exposed) and 0.26 pg/g lipid (controls)	Prostate specific antigen	↔
	Serum geometric mean 1,2,3,4,6,7,8-heptaCDF 0.41 pg/g lipid (exposed) and 0.04 pg/g lipid (controls)	Prostate specific antigen	↔
	Serum geometric mean 1,2,3,4,7,8,9-heptaCDF 0.04 pg/g lipid (exposed) and 0.03 pg/g lipid (controls)	Prostate specific antigen	↔
	Serum geometric mean octaCDF 0.0023 pg/g lipid (exposed) and 0.0020 pg/g lipid (controls)	Prostate specific antigen	↔
Sun et al. 2017 Cross-sectional, 50 men living in areas highly exposed to Agent Orange and 48 controls (Vietnam)	Serum geometric mean 1,2,3,4,7,8-hexaCDF 32.8 pg/g lipid (exposed) and 3.9 pg/g lipid (controls)	Testosterone	↔
		DHT	↔
		DHEA	↔
		Estradiol	↔
		3β-HSD	↔
	Serum geometric mean 1,2,3,6,7,8-hexaCDF 24.9 pg/g lipid (exposed) and 4.4 pg/g lipid (controls)	Testosterone	↔
		DHT	↔
		DHEA	↔
		Estradiol	↔
		3β-HSD	↔
	Serum geometric mean 1,2,3,4,6,7,8-heptaCDF 47.4 pg/g lipid (exposed) and 4.2 pg/g lipid (controls)	Testosterone	↔
		DHT	↔
		DHEA	↔
		Estradiol	↔
		3β-HSD	↔
Tsukimori et al. 2008 Retrospective, 214 women in the Yusho cohort who gave birth prior to exposure (152 women, 204 births), during 1968–1977 (69 women, 122 births), 1978–1987 (21 women, 88 births), and 1988–2003 (15 women, 98 births) (Japan)	Serum estimated geometric mean 2,3,4,7,8-pentaCDF • Prior to exposure 7.25 pg/g lipid (general population levels) • 1968–1977 2899.3 pg/g lipid • 1978–1987 697.7 pg/g lipid • 1988–2003 39.5 pg/g lipid	Induced abortion	↑
		Spontaneous abortion	↑
		Preterm delivery (1968–1977)	↑
		Pregnancy loss	↑

Table 2-7. Results of Epidemiological Studies Evaluating Exposure to CDFs and Reproductive Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Van Luong et al. 2018 Cross-sectional, 42 men living in areas highly exposed to Agent Orange (Vietnam)	Serum 2,3,7,8-tetraCDF geometric mean 7.0 pg/g lipid	FSH	↔
		LH	↔
		Progesterone	↔
		Prolactin	↔
		Estradiol	↔
		Testosterone	↓
	Serum 1,2,3,7,8-pentaCDF geometric mean 4.8 pg/g lipid	FSH	↔
		LH	↔
		Progesterone	↔
		Prolactin	↔
		Estradiol	↔
		Testosterone	↓
	Serum 2,3,4,7,8-pentaCDF geometric mean13.2 pg/g lipid	FSH	↔
		LH	↔
		Progesterone	↔
		Prolactin	↔
		Estradiol	↔
		Testosterone	↔
	Serum 1,2,3,4,7,8-hexaCDF geometric mean 17.3 pg/g lipid	FSH	↔
		LH	↔
		Progesterone	↔
		Prolactin	↔
		Estradiol	↔
		Testosterone	↔
	Serum 1,2,3,6,7,8-hexaCDF geometric mean 12.8 pg/g lipid	FSH	↔
		LH	↔
		Progesterone	↔
		Prolactin	↑
		Estradiol	↓
		Testosterone	↔
	Serum 1,2,3,7,8,9-hexaCDF geometric mean 4.9 pg/g lipid	FSH	↔
		LH	↔
		Progesterone	↔
		Prolactin	↔
		Estradiol	↔
		Testosterone	↔

Table 2-7. Results of Epidemiological Studies Evaluating Exposure to CDFs and Reproductive Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Serum 2,3,4,6,7,8-hexaCDF geometric mean 5.5 pg/g lipid	FSH	↔
		LH	↔
		Progesterone	↔
		Prolactin	↔
		Estradiol	↔
		Testosterone	↓
	Serum 1,2,3,4,6,7,8-heptaCDF geometric mean 17.6 pg/g lipid	FSH	↔
		LH	↔
		Progesterone	↔
		Prolactin	↔
		Estradiol	↓
		Testosterone	↓
	Serum 1,2,3,4,7,8,9-heptaCDF geometric mean 6.1 pg/g lipid	FSH	↔
		LH	↔
		Progesterone	↔
		Prolactin	↔
		Estradiol	↔
		Testosterone	↓
	Serum octaCDF geometric mean 16.7 pg/g lipid	FSH	↔
		LH	↔
		Progesterone	↔
		Prolactin	↔
		Estradiol	↔
		Testosterone	↓

↑ = association between biomarker level and outcome; ↓ = inverse association between biomarker level and outcome; ↔ = no association between biomarker level and outcome; A-dione = androstenedione; CDF = chlorodibenzofuran; DHEA = dehydroepiandrosterone; DHT = dihydrotestosterone; FSH = follicle stimulating hormone; 3β-HSD = 3β-hydroxysteroid dehydrogenase; LH = luteinizing hormone

Irregular menstrual cycles and abnormal basal body temperature patterns were observed in ≈60 and 85% of female Yusho cohort members, respectively (Kusuda 1971). These alterations were accompanied by decreased urinary excretion of estrogens, pregnanediol, and pregnanetriol, and possibly suggest corpus luteum insufficiency and retarded follicular maturation. In a follow-up study, Yusho cohort members reported an increased prevalence of abnormal uterine bleeding, menorrhagia, and hypomenorrhea (Akahane et al. 2018). A study of the Yu-Cheng cohort found slightly shorter menstrual cycle lengths, with a greater reduction among women with skin lesions (Yang et al. 2011). No effects on menstrual

cycle irregularity or dysmenorrhea were observed. However, among women exposed prior to menarche, a reduction in cycle length and longer days of menstrual flow were observed. Two general population studies examined the possible relationship between CDF exposure and risk of endometriosis (Table 2-7). Martínez-Zamora et al. (2015) found higher serum 2,3,4,7,8-pentaCDF levels among women with deep infiltrating endometriosis; the study also found associations for two CDD congeners (2,3,7,8-TCDD and 1,2,3,7,8-pentaCDD). The second study found no differences in total serum CDF levels between women with endometriosis and those without endometriosis (Cai et al. 2011).

An increase in self-reported male impotency was found among men in the Yusho cohort (Akahane et al. 2018). Hsu et al. (2016) reported an increase in abnormal sperm morphology in the Yu-Cheng cohort; there were no alterations in semen volume, sperm count, or percentage of motile sperm. The study also reported a nonsignificant increase in the ratio of normal X to normal Y sperm. Three studies of Vietnamese men living in areas sprayed with Agent Orange examined potential male reproductive effects associated with elevated levels of CDF congeners (Table 2-7); it is noted that Agent Orange was contaminated with 2,3,7,8-TCDD and the observed effects may be due to dioxin exposure. Van Luong et al. (2018) reported inverse associations between serum testosterone levels and serum 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, 2,3,4,6,7,8-hexaCDF, 1,2,3,4,6,7,8-heptaCDF, 1,2,3,4,7,8,9-heptaCDF, and octaCDF levels; inverse associations between serum estradiol levels and serum 1,2,3,6,7,8-hexaCDF, and 1,2,3,4,6,7,8-heptaCDF levels; and an association between serum prolactin levels and 1,2,3,6,7,8-hexaCDF and 1,2,3,4,6,7,8-heptaCDF. In contrast, Sun et al. (2017) found no associations between CDF congener levels and steroid hormone levels (testosterone, dihydrotestosterone, dehydroepiandrosterone, estradiol, and 3β-hydroxysteroid dehydrogenase). The third study found no associations between prostate specific antigen (PSA) levels and individual CDF congener levels (Sun et al. 2016). In a study of adult males in China, higher dehydroepiandrosterone (DHEA) levels were observed in the low CDFs-TEQ group compared to control, but it was not different in the moderate or high group (Shi et al. 2020). Higher androstenedione levels were observed in the high CDFs-TEQ group compared to control, but it was not different in the low or moderate groups.

There is limited epidemiological information on the potential effect of CDFs on fertility. Tsukimori et al. (2008) found an increased risk of induced abortions and preterm deliveries in the 10-year period after the Yusho incident but did not find alterations in the next two 10-year periods. The study also found associations between serum pentaCDF levels and induced abortion, spontaneous abortion, preterm delivery, and pregnancy loss (Table 2-7). A study of Yu-Cheng women found a prolonged time to pregnancy and reduced fertility (Yang et al. 2008).

There is limited information on the potential reproductive toxicity of CDFs in laboratory animals.

2,3,7,8-TetraCDF. Hypocellularity of the seminiferous tubules was observed in guinea pigs given single lethal gavage doses of ≥10 µg/kg/day 2,3,7,8-tetraCDF (Moore et al. 1979).

1,2,3,4,8-PentaCDF. Histology of the testis, ovary, and uterus was normal in rats administered dietary dosages of ≤600 µg/kg/day 1,2,3,4,8-pentaCDF for 13 weeks (Pluess et al. 1988a).

1,2,3,7,8-PentaCDF. A 13-week dietary exposure to ≤20 µg/kg/day did not result in histological alterations in the testis, ovary, or uterus of rats (Pluess et al. 1988a).

2,3,4,7,8-PentaCDF. A single lethal gavage dose of ≥10 µg/kg 2,3,4,7,8-pentaCDF resulted in hypocellularity of the seminiferous tubules in guinea pigs (Moore et al. 1979). There were no testicular histological changes in rats treated with a single gavage dose of ≤2,000 µg/kg 2,3,4,7,8-pentaCDF (Brewster et al. 1988). No histological alterations were observed in the testis, ovary, or uterus of rats exposed to ≤20 µg/kg/day 2,3,4,7,8-pentaCDF in the diet for 13 weeks (Pluess et al. 1988b), and no uterine alterations were observed in rats administered 0.2 µg/kg 2,3,4,7,8-pentaCDF 5 days/week for 14 or 31 weeks (NTP 2006). However, in a chronic gavage study, squamous metaplasia was observed in the uterus of rats administered ≥0.044 µg/kg 2,3,4,7,8-pentaCDF for 2 years (NTP 2006).

Mixed Congeners. Dietary exposure to an uncharacterized mixture of two tetra-, four penta-, and four hexaCDFs for 4 weeks caused increased testes weight at ≥97 µg/kg/day and decreased seminal vesicle and ventral prostate weights and decreased testicular testosterone concentration at 960 µg/kg/day in Sprague-Dawley rats (Oishi et al. 1978). The apparent increase in testes weight may be due to concurrent depression of total body weight.

Summary. Irregular menstrual cycles have been reported among the Yusho and Yu-Cheng cohorts. Histological alterations have not been reported in the reproductive tissues of animals exposed to nonlethal doses for acute or intermediate durations. Uterine lesions were observed in rats chronically exposed to 2,3,4,7,8-pentaCDF. No animal studies evaluated reproductive function.

2.17 DEVELOPMENTAL

A number of developmental effects were reported in children born to mothers in the Yusho or Yu-Cheng cohorts, and associations between CDF levels and developmental outcomes were observed in children living in areas of Vietnam that were sprayed with Agent Orange and in general population studies. It is noted that communities living in Agent Orange contaminated areas were likely exposed to high levels of CDDs, particularly 2,3,7,8-TCDD. Studies that evaluated possible associations between maternal serum or breast milk CDF levels and health outcomes are summarized in Table 2-8; effects examined include birth outcome and growth (Konishi et al. 2009; Tawara et al. 2009; Van Tung et al. 2016; Vartiainen et al. 1998; Wang et al. 2019), endocrine (Nagayama et al. 2007; Oanh et al. 2018), neurodevelopmental (Huisman et al. 1995; Li et al. 2015a; Nakajima et al. 2006, 2017; Nguyen et al. 2018; Nishijo et al. 2012; Tai et al. 2013), and other effects (Pluim et al. 1994). Observed developmental effects included skin lesions, alterations in birth outcome, neurodevelopmental delays, altered reproductive system development, and altered immune system development. Skin lesions were commonly observed in children born to mothers in the Yusho or Yu-Cheng cohorts. The dermal changes were consistent with those observed in exposed adults (see Section 2.11) and include hyperpigmentation of the skin, nails, and gingivae; deformed nails; conjunctivitis; and acne (Funatsu et al. 1971; Gladen et al. 1990; Hsu et al. 1985, 1993; Rogan et al. 1988; Taki et al. 1969; Yamaguchi et al. 1971; Yoshimura 1974). These effects generally diminished as the babies grew older.

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Huisman et al. 1995 Prospective, 418 mother-infant pairs (Netherlands)	Breast milk median 2,3,7,8-tetraCDF 0.73 mg/kg lipid	Neurological optimality score	↑
	Breast milk median 1,2,3,7,8-pentaCDF 0.09 mg/kg lipid	Neurological optimality score	↔
	Breast milk median 2,3,4,7,8-pentaCDF 6.48 mg/kg lipid	Neurological optimality score	↑
	Breast milk median 1,2,3,4,7,8-hexaCDF 5.59 mg/kg lipid	Neurological optimality score	↔
	Breast milk median 1,2,3,6,7,8-hexaCDF 0.08 mg/kg lipid	Neurological optimality score	↔

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Breast milk median 1,2,3,7,8,9-hexaCDF mg/kg lipid	Neurological optimality score	↔
	Breast milk median 2,3,4,6,7,8-hexaCDF 3.00 mg/kg lipid	Neurological optimality score	↔
	Breast milk median 1,2,3,4,6,7,8-heptaCDF 6.32 mg/kg lipid	Neurological optimality score	↔
	Breast milk median 1,2,3,4,7,8,9-heptaCDF 0.19 mg/kg lipid	Neurological optimality score	↔
	Breast milk median octaCDF 0.38 mg/kg lipid	Neurological optimality score	↔
Konishi et al. 2009 Prospective, 514 mother-infant pairs (Japan)	Maternal serum mean total CDFs 20.5 pg/g lipid	Birth weight	↓
	Maternal serum mean 2,3,7,8-tetraCDF not reported	Birth weight	↔
	Maternal serum mean 2,3,4,7,8-pentaCDF not reported	Birth weight	↓
	Maternal serum mean 1,2,3,4,7,8-hexaCDF not reported	Birth weight	↔
	Maternal serum mean 1,2,3,6,7,8-hexaCDF not reported	Birth weight	↔
	Maternal serum mean 1,2,3,4,6,7,8-heptaCDF not reported	Birth weight	↔
Li et al. 2015b Retrospective, 53 prenatally exposed adults (21 years of age) (Yu-Cheng) and 87 referents (Taiwan)	Estimated serum median 2,3,4,7,8-pentaCDF 1298.72 pg/g lipid	Hearing threshold at 250, 500, and 1,000 Hz in right ear and 500 and 4,000 Hz in left ear	↑
		Average hearing threshold in right and left ears	↑
		DPOAE at 1.5 and 2 Hz in right ear	↓
		Average DPOAE in right and left ears	↓

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Li et al. 2018 Nested case-control, 28 mothers of boys with cryptorchidism, 30 mothers of healthy boys (Denmark)	Placental geometric mean 2,3,7,8-tetraCDF 0.27 ng/g lipid	T4	↔
		T3	↑
		rT3	↔
	Placental geometric mean 1,2,3,7,8-pentaCDF 0.23 ng/g lipid	T4	↔
		T3	↑
		rT3	↑
	Placental geometric mean 2,3,4,7,8-pentaCDF 7.88 ng/g lipid	T4	↔
		T3	↔
		rT3	↔
	Placental geometric mean 1,2,3,4,7,8-hexaCDF 2.08 ng/g lipid	T4	↔
		T3	↔
		rT3	↔
	Placental geometric mean 1,2,3,6,7,8-hexaCDF 1.23 ng/g lipid	T4	↔
		T3	↔
		rT3	↔
	Placental geometric mean 2,3,4,6,7,8-hexaCDF 0.47 ng/g lipid	T4	↔
		T3	↔
		rT3	↑
	Placental geometric mean 1,2,3,4,6,7,8-heptaCDF 96.6 ng/g lipid	T4	↑
		T3	↔
		rT3	↔
Nagayama et al. 2007 Case-control, 34 case (22 neonates diagnosed with cretinism, 4 with hyper-TSH-emia, and 4 negative in re-evaluation) and 102 controls (Japan)	Breast milk mean total CDF TEQ[a] 0.16 pg/g lipid (infants with cretinism), 0.09 pg/g lipid (hyper-TSH-emia), 0.09 pg/g lipid (negative), and 0.06 pg/g lipid (control)	Induction of cretinism	↑
Nakajima et al. 2006 Prospective, 134 mother-infant (6 months of age) pairs (Japan)	Maternal serum mean 2,3,7,8-tetraCDF 0.7 pg/g lipid	MDI	↔
		PDI	↓
	Maternal serum mean 1,2,3,7,8-pentaCDF 0.6 pg/g lipid	MDI	↔
		PDI	↓
	Maternal serum mean 2,3,4,7,8-pentaCDF 6.5 pg/g lipid	MDI	↔
		PDI	↔
	Maternal serum mean 1,2,3,4,7,8-hexaCDF 2.6 pg/g lipid	MDI	↔
		PDI	↔

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Maternal serum mean 1,2,3,6,7,8-hexaCDF 3.0 pg/g lipid	MDI	↔
		PDI	↓
	Maternal serum mean 2,3,4,6,7,8-hexaCDF 1.1 pg/g lipid	MDI	↔
		PDI	↔
	Maternal serum mean 1,2,3,4,6,7,8-heptaCDF 3.1 pg/g lipid	MDI	↔
		PDI	↔
	Maternal serum mean octaCDF 2.1 pg/g lipid	MDI	↔
		PDI	↔
	Maternal serum mean total CDF TEQ[a] 4.2 pg/g lipid	MDI	↔
		PDI	↔
Nakajima et al. 2017 Prospective, mother-infant pairs (190 pairs at 6 months of age and 121 pairs at 18 months of age) (Japan)	Maternal serum 75th percentile 2,3,7,8-tetraCDF 1.1 pg/g lipid	MDI at 6 months	↔
		PDI at 6 months	↓ (male infants only)
		MDI at 18 months	↔
		PDI at 18 months	↔
	Maternal serum 1,2,3,7,8-pentaCDF not detected below 75th percentile	MDI at 6 months	↔
		PDI at 6 months	↓ (male infants only)
		MDI at 18 months	↔
		PDI at 18 months	↔
	Maternal serum median 2,3,4,7,8-pentaCDF 5.8 pg/g lipid	MDI at 6 months	↔
		PDI at 6 months	↔
		MDI at 18 months	↔
		PDI at 18 months	↔
	Maternal serum median 1,2,3,4,7,8-hexaCDF 2.5 pg/g lipid	MDI at 6 months	↔
		PDI at 6 months	↔
		MDI at 18 months	↔
		PDI at 18 months	↔
	Maternal serum median 1,2,3,6,7,8-hexaCDF 2.7 pg/g lipid	MDI at 6 months	↔
		PDI at 6 months	↔
		MDI at 18 months	↔
		PDI at 18 months	↔
	Maternal serum 2,3,4,6,7,8-hexaCDF not detected below 75th percentile	MDI at 6 months	↔
		PDI at 6 months	↔
		MDI at 18 months	↔
		PDI at 18 months	↔
		MDI at 6 months	↔
		PDI at 6 months	↔

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Maternal serum 1,2,3,7,8,9-hexaCDF not detected below 75th percentile	MDI at 18 months	↔
		PDI at 18 months	↔
	Maternal serum median 1,2,3,4,6,7,8-heptaCDF 2.4 pg/g lipid	MDI at 6 months	↔
		PDI at 6 months	↔
		MDI at 18 months	↓ (female infants only)
		PDI at 18 months	↔
	Maternal serum octaCDF not detected below 75th percentile	MDI at 6 months	↔
		PDI at 6 months	↔
		MDI at 18 months	↔
		PDI at 18 months	↔
	Maternal serum median total CDF 19.7 pg/g lipid	MDI at 6 months	↔
		PDI at 6 months	↔
		MDI at 18 months	↔
		PDI at 18 months	↔
Nguyen et al. 2018 Prospective, 185 mother-infant (3 years old) pairs (106 male infants and 79 female infants) living in areas highly exposed to Agent Orange (Vietnam)	Breast milk median 2,3,7,8-tetraCDF 0.5 pg/g lipid male infants and 0.6 pg/g lipid female infants	Tests of eating behavior	↔
	Breast milk median 1,2,3,7,8-pentaCDF 1.2 pg/g lipid male infants and 1.3 pg/g lipid female infants	Tests of eating behavior	↔
	Breast milk median 2,3,4,7,8-pentaCDF 7.2 pg/g lipid male infants and 8.1 pg/g lipid female infants	Tests of eating behavior	↔
	Breast milk median 1,2,3,4,7,8-pentaCDF 16.5 pg/g lipid male infants and 19.9 pg/g lipid female infants	Tests of eating behavior	↔
	Breast milk median 1,2,3,6,7,8-hexaCDF 10.8 pg/g lipid male infants and 12.0 pg/g lipid female infants	Tests of eating behavior	↔
	Breast milk median 1,2,3,7,8,9-hexaCDF 0.2 pg/g lipid male infants and 0.3 pg/g lipid female infants	Tests of eating behavior	↓ enjoyment of food test ↔ for four other tests

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Breast milk median 1,2,3,7,8,9-hexaCDF 0.2 pg/g lipid male infants and 0.3 pg/g lipid female infants	Tests of eating behavior	↔
	Breast milk median 2,3,4,6,7,8-hexaCDF 1.3 pg/g lipid male infants and 1.5 pg/g lipid female infants	Tests of eating behavior	↔
	Breast milk median 1,2,3,4,6,7,8-heptaCDF 10.8 pg/g lipid male infants and 13.5 pg/g lipid female infants	Tests of eating behavior	↔
	Breast milk median 1,2,3,4,7,8,9-heptaCDF 1.2 pg/g lipid male infants and 1.6 pg/g lipid female infants	Tests of eating behavior	↓ enjoyment of food test ↔ for four other tests
	Breast milk median octaCDF 0.5 pg/g lipid male infants and 0.5 pg/g lipid female infants	Tests of eating behavior	↓ enjoyment of food, food responsiveness, and food approach tests ↔ for two other tests
	Breast milk median total CDF TEQ[b] 5.2 pg/g lipid male infants and 5.7 pg/g lipid female infants	Tests of eating behavior	↔
Nishijo et al. 2012 Prospective, 122 mother-infant (3 years old) pairs living in areas highly exposed to Agent Orange (Vietnam)	Breast milk 2,3,7,8-tetraCDF levels not reported	Autism spectrum tests	↓ Atypical language score ↔ other autism tests
	Breast milk 1,2,3,7,8-pentaCDF levels not reported	Autism spectrum tests	↑ Social communication and social/ emotional reciprocity scores ↔ other autism tests
	Breast milk 2,3,4,7,8-pentaCDF levels not reported	Autism spectrum tests	↔
	Breast milk 1,2,3,4,7,8-hexaCDF levels not reported	Autism spectrum tests	↔
	Breast milk 1,2,3,6,7,8-hexaCDF levels not reported	Autism spectrum tests	↔
	Breast milk 1,2,3,7,8,9-hexaCDF levels not reported	Autism spectrum tests	↔

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Breast milk 2,3,4,6,7,8-hexaCDF levels not reported	Autism spectrum tests	↓ Attention/self-regulation score ↔ other autism tests
	Breast milk 1,2,3,4,6,7,8-heptaCDF levels not reported	Autism spectrum tests	↔
	Breast milk 1,2,3,4,7,8,9-heptaCDF levels not reported	Autism spectrum tests	↓ Attention/self-regulation score ↔ other autism tests
	Breast milk octaCDF levels not reported	Autism spectrum tests	↔
Oanh et al. 2018 Prospective, 35 mother-infant (5 years old) pairs living in areas highly exposed to Agent Orange and 50 mother-infant controls (Vietnam)	Breast milk geometric mean 2,3,7,8-tetraCDF 0.6 pg/g lipid in hot spot and 0.6 pg/g lipid in control area	Androstenedione	↑
		DHEA	↔
		Testosterone	↔
	Breast milk geometric mean 1,2,3,7,8-pentaCDF 1.8 pg/g lipid in hot spot and 0.4 pg/g lipid in control area	Androstenedione	↔
		DHEA	↓
		Testosterone	↓
	Breast milk geometric mean 2,3,4,7,8-pentaCDF 5.6 pg/g lipid in hot spot and 3.0 pg/g lipid in control area	Androstenedione	↑
		DHEA	↓
		Testosterone	↓
	Breast milk geometric mean 1,2,3,4,7,8-hexaCDF 13.2 pg/g lipid in hot spot and 1.9 pg/g lipid in control area	Androstenedione	↑
		DHEA	↓
		Testosterone	↓
	Breast milk geometric mean 1,2,3,6,7,8-hexaCDF 7.7 pg/g lipid in hot spot and 1.6 pg/g lipid in control area	Androstenedione	↑
		DHEA	↓
		Testosterone	↓
	Breast milk geometric mean 1,2,3,7,8,9-hexaCDF 0.3 pg/g lipid in hot spot and 0.1 pg/g lipid in control area	Androstenedione	↑
		DHEA	↓
		Testosterone	↔
	Breast milk geometric mean 2,3,4,6,7,8-hexaCDF 1.3 pg/g lipid in hot spot and 0.5 pg/g lipid in control area	Androstenedione	↑
		DHEA	↓
		Testosterone	↓
	Breast milk geometric mean 1,2,3,4,6,7,8-heptaCDF 14.0 pg/g lipid in hot spot and 1.4 pg/g lipid in control area	Androstenedione	↑
		DHEA	↓
		Testosterone	↓

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Breast milk geometric mean 1,2,3,4,7,8,9-heptaCDF 1.4 pg/g lipid in hot spot and 0.2 pg/g lipid in control area	Androstenedione	↑
		DHEA	↓
		Testosterone	↓
	Breast milk geometric mean octaCDF 1.0 pg/g lipid in hot spot and 0.3 pg/g lipid in control area	Androstenedione	↑
		DHEA	↔
		Testosterone	↔
	Breast milk geometric mean total CDF TEQ[b] 4.3 pg/g lipid in hot spot and 1.4 pg/g lipid in control area	Androstenedione	↑
		DHEA	↓
		Testosterone	↓
Oyama et al. 2021 Prospective, 60 mother-child (7 years old) pairs living in areas highly exposed to Agent Orange and 63 mother-child controls (Vietnam)	Breast milk geometric mean 1,2,3,7,8-pentaCDF 1.7 pg/g lipid in hot spot and 0.4 pg/g lipid in control area	Progesterone	↔ male children ↑ female children
		DHEA	↑ male children ↔ female children
		Testosterone	↓ male children ↔ female children
		3β-HSD	↓ male children ↔ female children
		17β-HSD	↓ male children ↔ female children
		CYP17 lyase	↑ male children ↔ female children
	Breast milk geometric mean 2,3,4,7,8-pentaCDF 5.5 pg/g lipid in hot spot and 2.9 pg/g lipid in control area	Progesterone	↑ male children ↔female children
		DHEA	↑ male children ↔ female children
		Testosterone	↓ male children ↔ females
		3β-HSD	↓ male children ↔ female children
		17β-HSD	↓ male children ↔ female children
		CYP17 lyase	↑ male children ↔ female children
	Breast milk geometric mean 1,2,3,4,7,8-hexaCDF 12.5 pg/g lipid in hot spot and 1.8 pg/g lipid in control area	Progesterone	↑ male children ↑ female children
		DHEA	↑ male children ↔ female children
		Testosterone	↓ male children ↔ female children

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		3β-HSD	↓ male children ↔ female children
		17β-HSD	↓ male children ↔ female children
		CYP17 lyase	↑ male children ↔ female children
	Breast milk geometric mean 1,2,3,6,7,8-hexaCDF 7.5 pg/g lipid in hot spot and 1.6 pg/g lipid in control area	Progesterone	↑ male children ↑ female children
		DHEA	↑ male children ↔ female children
		Testosterone	↓ male children ↔ female children
		3β-HSD	↓ male children ↔ female children
		17β-HSD	↓ male children ↔ female children
		CYP17 lyase	↑ male children ↔ female children
	Breast milk geometric mean 1,2,3,7,8,9-hexaCDF 0.3 pg/g lipid in hot spot and 0.1 pg/g lipid in control area	Progesterone	↔ male children ↑ female children
		DHEA	↑ male children ↔ female children
		Testosterone	↓ male children ↔ female children
		3β-HSD	↓ male children ↔ female children
		17β-HSD	↓ male children ↔ female children
		CYP17 lyase	↑ male children ↔ female children
	Breast milk geometric mean 2,3,4,6,7,8-hexaCDF 1.3 pg/g lipid in hot spot and 0.5 pg/g lipid in control area	Progesterone	↔ male children ↑ female children
		DHEA	↑ male children ↔ female children
		Testosterone	↓ male children ↔ female children
		3β-HSD	↓ male children ↔ female children
		17β-HSD	↓ male children ↔ female children

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		CYP17 lyase	↑ male children ↔ female children
	Breast milk geometric mean 1,2,3,4,6,7,8-heptaCDF 13.1 pg/g lipid in hot spot and 1.4 pg/g lipid in control area	Progesterone	↑ male children ↔ female children
		DHEA	↑ male children ↔ female children
		Testosterone	↓ male children ↔ female children
		3β-HSD	↓ male children ↓ female children
		17β-HSD	↓ male children ↔ female children
		CYP17 lyase	↑ male children ↔ female children
	Breast milk geometric mean 1,2,3,4,7,8,9-heptaCDF 1.3 pg/g lipid in hot spot and 0.2 pg/g lipid in control area	Progesterone	↑ male children ↑ female children
		DHEA	↑ male children ↔ female children
		Testosterone	↓ male children ↔ female children
		3β-HSD	↓ male children ↔ female children
		17β-HSD	↓ male children ↔ female children
		CYP17 lyase	↑ male children ↔ female children
	Breast milk geometric mean octaCDF 0.9 pg/g lipid in hot spot and 0.3 pg/g lipid in control area	Progesterone	↔ male children ↔ female children
		DHEA	↑ male children ↔ female children
		Testosterone	↓ male children ↔ female children
		3β-HSD	↓ male children ↔ female children
		17β-HSD	↓ male children ↔ female children
		CYP17 lyase	↑ male children ↔ female children

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Pham et al. 2020 Cross-sectional, 172 mother-child pairs (100 male children and 72 female children) living in areas highly exposed to Agent Orange (Vietnam)	Breast milk 2,3,7,8-tetraCDF (levels not reported)	Salivary testosterone	↔ male children ↓ female children
		Gaze behavior	↔ male children ↔ female children
	Breast milk 1,2,3,7,8-pentaCDF (levels not reported)	Salivary testosterone	↔ male children ↔ female children
		Gaze behavior	↔ male children ↔ female children
	Breast milk 2,3,4,7,8-pentaCDF (levels not reported)	Salivary testosterone	↔ male children ↓ female children
		Gaze behavior	↔ male children ↔ female children
	Breast milk 1,2,3,4,7,8-hexaCDF (levels not reported)	Salivary testosterone	↔ male children ↔ female children
		Gaze behavior	↔ male children ↔ female children
	Breast milk 1,2,3,6,7,8-hexaCDF (levels not reported)	Salivary testosterone	↔ male children ↔ female children
		Gaze behavior	↔ male children ↔ female children
	Breast milk 1,2,3,7,8,9-hexaCDF (levels not reported)	Salivary testosterone	↔ male children ↓ female children
		Gaze behavior	↔ male children ↔ female children
	Breast milk 2,3,4,6,7,8-hexaCDF (levels not reported)	Salivary testosterone	↔ male children ↓ female children
		Gaze behavior	↔ male children ↔ female children
	Breast milk 1,2,3,4,6,7,8-heptaCDF (levels not reported)	Salivary testosterone	↔ male children ↔ female children
		Gaze behavior	↔ male children ↔ female children
	Breast milk 1,2,3,4,7,8,9-hexaCDF (levels not reported)	Salivary testosterone	↔ male children ↔ female children
		Gaze behavior	↔ male children ↔ female children
	Breast milk octaCDF (levels not reported)	Salivary testosterone	↔ male children ↔ female children
		Gaze behavior	↔ male children ↔ female children

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Pluim et al. 1994 Prospective, 6–8 mother-infant (11 weeks old) pairs (Netherlands)	Breast milk 2,3,7,8-tetraCDF levels not reported	Vitamin K levels	↓
		PIVKA-II levels	↔
	Breast milk 2,3,4,7,8-pentaCDF levels not reported	Vitamin K levels	↔
		PIVKA-II levels	↔
	Breast milk 1,2,3,4,7,8-hexaCDF levels not reported	Vitamin K levels	↔
		PIVKA-II levels	↔
	Breast milk 1,2,3,6,7,8-hexaCDF levels not reported	Vitamin K levels	↔
		PIVKA-II levels	↑
	Breast milk 1,2,3,7,8,9-hexaCDF levels not reported	Vitamin K levels	↔
		PIVKA-II levels	↔
	Breast milk 1,2,3,4,6,7,8-heptaCDF levels not reported	Vitamin K levels	↓
		PIVKA-II levels	↔
	Breast milk octaCDF levels not reported	Vitamin K levels	↔
		PIVKA-II levels	↔
Tai et al. 2013 Prospective, 158 mother-infant (3 years old) pairs living in areas highly exposed to Agent Orange (Vietnam)	Breast milk 2,3,7,8-tetraCDF levels not reported	Cognitive composite score	↔
		Receptive score	↔
		Expressive communication	↔
		Language composite score	↔
		Fine motor	↔
		Gross motor	↔
		Motor composite score	↔
	Breast milk 1,2,3,7,8-pentaCDF levels not reported	Cognitive composite score	↔
		Receptive score	↓
		Expressive communication	↓
		Language composite score	↓
		Fine motor	↓
		Gross motor	↔
		Motor composite score	↓

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Breast milk 2,3,4,7,8-pentaCDF levels not reported	Cognitive composite score	↔
		Receptive score	↔
		Expressive communication	↔
		Language composite score	↔
		Fine motor	↔
		Gross motor	↔
		Motor composite score	↔
	Breast milk 1,2,3,4,7,8-hexaCDF levels not reported	Cognitive composite score	↔
		Receptive score	↓
		Expressive communication	↔
		Language composite score	↓
		Fine motor	↔
		Gross motor	↔
		Motor composite score	↔
	Breast milk 1,2,3,6,7,8-hexaCDF levels not reported	Cognitive composite score	↔
		Receptive score	↓
		Expressive communication	↔
		Language composite score	↓
		Fine motor	↔
		Gross motor	↔
		Motor composite score	↔
	Breast milk 1,2,3,7,8,9-hexaCDF levels not reported	Cognitive composite score	↔
		Receptive score	↓
		Expressive communication	↓
		Language composite score	↓
		Fine motor	↔
		Gross motor	↔
		Motor composite score	↔

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Breast milk 2,3,4,6,7,8-hexaCDF levels not reported	Cognitive composite score	↔
		Receptive score	↓
		Expressive communication	↔
		Language composite score	↓
		Fine motor	↔
		Gross motor	↔
		Motor composite score	↔
	Breast milk 1,2,3,4,6,7,8-heptaCDF levels not reported	Cognitive composite score	↔
		Receptive score	↓
		Expressive communication	↔
		Language composite score	↓
		Fine motor	↔
		Gross motor	↔
		Motor composite score	↔
	Breast milk 1,2,3,4,7,8,9-heptaCDF levels not reported	Cognitive composite score	↔
		Receptive score	↔
		Expressive communication	↔
		Language composite score	↔
		Fine motor	↔
		Gross motor	↔
		Motor composite score	↔
	Breast milk octaCDF levels not reported	Cognitive composite score	↔
		Receptive score	↔
		Expressive communication	↔
		Language composite score	↔
		Fine motor	↔
		Gross motor	↔
		Motor composite score	↔

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Breast milk total CDF TEQ[b], levels not reported	Cognitive composite score	↔
		Receptive score	↔
		Expressive communication	↔
		Language composite score	↔
		Fine motor	↔
		Gross motor	↔
		Motor composite score	↔
Tawara et al. 2009 Cross-sectional, 75 mother-infant pairs	Breast milk mean 2,3,7,8-tetraCDF 1.02 pg/g lipid	Birth length	↔
		Birth weight	↔
		Chest circumference	↔
		Head circumference	↔
		Gestational weeks	↔
	Breast milk mean 1,2,3,7,8-pentaCDF 0.29 pg/g lipid	Birth length	↔
		Birth weight	↔
		Chest circumference	↔
		Head circumference	↔
		Gestational weeks	↔
	Breast milk mean 2,3,4,7,8-pentaCDF 8.14 pg/g lipid	Birth length	↓
		Birth weight	↔
		Chest circumference	↔
		Head circumference	↔
		Gestational weeks	↔
	Breast milk mean 1,2,3,4,7,8-hexaCDF 2.25 pg/g lipid	Birth length	↔
		Birth weight	↔
		Chest circumference	↔
		Head circumference	↔
		Gestational weeks	↔
	Breast milk mean 1,2,3,6,7,8-hexaCDF 2.27 pg/g lipid	Birth length	↓
		Birth weight	↔
		Chest circumference	↔
		Head circumference	↔
		Gestational weeks	↔

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Breast milk mean 2,3,4,6,7,8-hexaCDF 1.46 pg/g lipid	Birth length	↓
		Birth weight	↔
		Chest circumference	↔
		Head circumference	↔
		Gestational weeks	↔
	Breast milk mean 1,2,3,7,8,9-hexaCDF 0.03 pg/g lipid	Birth length	↔
		Birth weight	↔
		Chest circumference	↔
		Head circumference	↔
		Gestational weeks	↔
	Breast milk mean 1,2,3,4,6,7,8-heptaCDF 1.19 pg/g lipid	Birth length	↔
		Birth weight	↔
		Chest circumference	↔
		Head circumference	↔
		Gestational weeks	↔
	Breast milk mean 1,2,3,4,7,8,9-heptaCDF 0.04 pg/g lipid	Birth length	↔
		Birth weight	↔
		Chest circumference	↔
		Head circumference	↔
		Gestational weeks	↔
	Breast milk mean octaCDF 0.55 pg/g lipid	Birth length	↔
		Birth weight	↔
		Chest circumference	↔
		Head circumference	↔
		Gestational weeks	↔
	Breast milk mean total CDF TEQ[a] 4.89 pg/g lipid	Birth length	↓
		Birth weight	↔
		Chest circumference	↔
		Head circumference	↔
		Gestational weeks	↔

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Van Tung et al. 2016 Prospective, 58 mother-infant pairs living in areas highly exposed to Agent Orange and 62 control pairs (Vietnam)	Breast milk mean 2,3,7,8-tetraCDF TEQ[b] 0.07 pg/g lipid in hot spot and 0.067 pg/g lipid in control area	Weight	
		• Birth	↔
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Height	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Head circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Chest circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
	Breast milk mean 1,2,3,7,8-pentaCDF TEQ[b] 0.065 pg/g lipid in hot spot and 0.014 pg/g lipid in control area	Weight	
		• Birth	↔
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Height	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Head circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Chest circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
	Breast milk mean 2,3,4,7,8-pentaCDF TEQ[b] 1.909 pg/g lipid in hot spot and 0.914 pg/g lipid in control area	Weight	
		• Birth	↓
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Height	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Head circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Chest circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Breast milk mean 1,2,3,4,7,8-hexaCDF TEQ[b] 1.592 pg/g lipid in hot spot and 0.196 pg/g lipid in control area	Weight	
		• Birth	↔
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Height	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Head circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Chest circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
	Breast milk mean 1,2,3,6,7,8-hexaCDF TEQ[b] 0.926 pg/g lipid in hot spot and 0.166 pg/g lipid in control area	Weight	
		• Birth	↔
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Height	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Head circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Chest circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
	Breast milk mean 1,2,3,7,8,9-hexaCDF TEQ[b] 0.041 pg/g lipid in hot spot and 0.014 pg/g lipid in control area	Weight	
		• Birth	↔
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Height	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Head circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Chest circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Breast milk mean 2,3,4,6,7,8-hexaCDF TEQ[b] 0.159 pg/g lipid in hot spot and 0.055 pg/g lipid in control area	Weight	
		• Birth	↔
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Height	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Head circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Chest circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
	Breast milk mean 1,2,3,4,6,7,8-heptaCDF TEQ[b] 0.183 pg/g lipid in hot spot and 0.015 pg/g lipid in control area	Weight	
		• Birth	↔
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Height	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Head circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Chest circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
	Breast milk mean 1,2,3,4,7,8,9-heptaCDF TEQ[b] 0.018 pg/g lipid in hot spot and 0.002 pg/g lipid in control area	Weight	
		• Birth	↔
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Height	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Head circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Chest circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Breast milk mean octaCDF TEQ[b] 0.001 pg/g lipid in hot spot and 0.000 pg/g lipid in control area	Weight	
		• Birth	↔
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Height	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Head circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Chest circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
	Breast milk mean total CDF TEQ[b] 4.965 pg/g lipid in hot spot and 1.442 pg/g lipid in control area	Weight	
		• Birth	↔
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Height	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Head circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
		Chest circumference	
		• 8–9 weeks	↔
		• 12–14 weeks	↔
Vartiainen et al. 1998 Cross-sectional, 167 mother-infant pairs (Finland)	Breast milk 2,3,7,8-tetraCDF, levels not reported	Birth weight	↓
	Breast milk 1,2,3,7,8-pentaCDF, levels not reported	Birth weight	↓
	Breast milk 2,3,4,7,8-pentaCDF, levels not reported	Birth weight	↓
Wang et al. 2019 Prospective, 27 mother-infant pairs living near electronic waste dismantling region and 35 controls (China)	Breast milk total CDF TEQ[b] 6.6 pg/g lipid in exposed and 2.1 pg/g lipid in controls	Height	
		• 6 months	↔
		• 3 years	↔
		Weight	
		• 6 months	↔
		• 3 years	↔
		BMI	
		• 6 months	↔
		• 3 years	↔

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
		Head circumference • 6 months • 3 years	 ↔ ↔
		Chest circumference • 6 months • 3 years	 ↔ ↔
Wang et al. 2022 Cross-sectional, 50 mother-child pairs living near an electronic waste area, follow-up at 6 years, 42 pairs remained (China)	Breast milk geometric mean 2,3,7,8-tetraCDF 5.1 pg/g lipid	DHEA	↔
		A-dione	↔
		Testosterone	↔
		Progesterone	↔
	Breast milk geometric mean 1,2,3,7,8-pentaCDF 3.5 pg/g lipid	DHEA	↔
		A-dione	↓
		Testosterone	↔
		Progesterone	↔
	Breast milk geometric mean 2,3,4,7,8-pentaCDF 8.5 pg/g lipid	DHEA	↓
		A-dione	↔
		Testosterone	↔
		Progesterone	↔
	Breast milk geometric mean 1,2,3,4,7,8-hexaCDF 3.3 pg/g lipid	DHEA	↔
		A-dione	↔
		Testosterone	↔
		Progesterone	↔
	Breast milk geometric mean 1,2,3,6,7,8-hexaCDF 5.2 pg/g lipid	DHEA	↓
		A-dione	↔
		Testosterone	↔
		Progesterone	↔
	Breast milk geometric mean 2,3,4,6,7,8-hexaCDF 3.7 pg/g lipid	DHEA	↔
		A-dione	↔
		Testosterone	↔
		Progesterone	↔
	Breast milk geometric mean 1,2,3,4,6,7,8-heptaCDF 6.5 pg/g lipid	DHEA	↔
		A-dione	↔
		Testosterone	↔
		Progesterone	↔
	Breast milk geometric mean Total CDF 4.6 pg/g lipid	DHEA	↓
		A-dione	↔
		Testosterone	↔
		Progesterone	↔

Table 2-8. Results of Epidemiological Studies Evaluating Exposure to CDFs and Developmental Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Yamazaki et al. 2022 Cross-sectional, 55 mother-child pairs from Sapporo Cohort, follow-up at 13 years (Japan)	Maternal blood median total CDF 2.58 TEQ pg/g lipid	Omission error rate	↔
		False alarm rate	↔
		Reaction time	↓ difficult test
		P3a latency	↔
		P3a amplitude	↓ difficult test
		P3b latency	↔
		P3b amplitude	↓ difficult test

[a]TEQs were calculated using the WHO 1998 TEF values (van den Berg et al. 1998).
[b]TEQs were calculated using the WHO 2005 TEF values (van den Berg et al. 2006).

↑ = association between biomarker level and outcome; ↓ = inverse association between biomarker level and outcome; ↔ = no association between biomarker level and outcome; BMI = body mass index; CDF = chlorodibenzofuran; DHEA = dehydroepiandrosterone; DPOAE = distortion product otoacoustic emissions; MDI = mental developmental index score; PDI = psychomotor developmental index score; rT3 = reverse triiodothyronine; T_3 = serum triiodothyronine; T4 = thyroxine; TEF = toxic equivalency factor; TEQ = toxic equivalency; TSH = thyroid stimulating hormone; WHO = World Health Organization

Decreased birth weight was another commonly reported effect in the Yusho and Yu-Cheng cohorts (Funatsu et al. 1971; Lan et al. 1987; Rogan 1989; Taki et al. 1969; Yamaguchi et al. 1971). A health survey of children of mothers in the Yu-Cheng cohort known to have been *in utero* during or after exposure found that mean birth weight was decreased ≈15% (Gladen et al. 1990; Rogan et al. 1988; Yen et al. 1994). The greatest decreases in birth weight were found in children born in the first year after the incident (Yen et al. 1994). Decreases in height and muscular development (as indicated by lower total lean tissue mass) were also observed in the children of the Yu-Cheng cohort; when grouped by birth order, these effects were only observed in the first child born after the incident (Guo et al. 1994a). The Guo et al. (1994b) study did not find alterations in weight or bone mineral density. An inverse association between birth weight and maternal serum total CDF levels and 2,3,4,7,8-pentaCDF was found in a general population study (Konishi et al. 2009); no associations were found for 2,3,7,8-tetraCDF, 1,2,3,4,7,8-hexaCDF, 1,2,3,6,7,8-hexaCDF, or 1,2,3,4,6,7,8-heptaCDF. Similarly, Vartianien et al. (1998) found inverse associations between birth weight and breast milk levels of 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, and 2,3,4,7,8-pentaCDF. In contrast, Tawara et al. (2009) found no association between breast milk levels of CDF congeners and birth weight, head circumference, or chest circumference in a general population study, but did find inverse associations between birth length and breast milk 2,3,4,7,8-pentaCDF, 1,2,3,6,7,8-hexaCDF, 2,3,4,6,7,8-hexaCDF, and total CDF TEQ levels. Two studies examined birth outcomes in populations living in highly contaminated areas. Van Tung et al.

(2016) found an inverse association between breast milk 2,3,4,7,8-pentaCDF TEQ and birth weight among a population living in an area of Vietnam that was sprayed with Agent Orange; no associations with birth weight were found for 2,3,7,8-tetraCDF TEQ, 1,2,3,7,8-pentaCDF TEQ, several hexaCDF and heptaCDF congener TEQs, octaCDF TEQ, or total CDF TEQ. The study also found no associations with weight, height, head circumference, or chest circumference at 8–9 or 12–14 weeks of age. Similarly, Wang et al. (2019) found no association between breast milk total CDF TEQ and height, weight, BMI, head circumference, or chest circumference at 6 months or 3 years of age among the children of women living near an electronic waste dismantling region in China. The results of the Konishi et al. (2009); Tawara et al. (2009), Van Tung et al. (2016), Vartiainen et al. (1998), and Wang et al. (2019) are presented in Table 2-8. No alterations in sex ratio were found in infants born in the Yusho affected area between 1968 and 1977 (Yoshimura et al. 2001). There is limited information on the occurrence of birth defects in the Yusho and Yu-Cheng cohorts. Wang et al. (2003) reported increases in the prevalence of dental defects (congenitally missing germ, neonatal teeth, and tooth rotation) in children of Yu-Cheng mothers. A study of Vietnamese children in an Agent Orange affected area reported higher levels of serum 1,2,3,4,8,9-hexaCDF in fathers of children with birth defects (Tawara et al. 2008). An association between breast milk levels of CDFs and the risk of cretinism was reported in a general population study (Nagayama et al. 2007). Inverse associations between vitamin K levels in 11-month-old infants and serum 1,2,3,7,8-pentaCDF and 1,2,3,4,6,7,8-heptaCDF levels were found in a general population study (Pluim et al. 1994). See Table 2-8 for a summary of the Nagayama et al. (2007) and Pluim et al. (1994) studies.

Neurobehavioral assessment based on parental reports showed that 49% of the children in the Yu-Cheng cohort were delayed in achieving developmental milestones compared to 22% of unexposed children, but this was not clearly corroborated by neurological examiners (Rogan et al. 1988; Yu et al. 1991). Cognitive testing (Bayley mental and psychomotor developmental indices, Stanford-Binet test, Wechsler Intelligence Scale for Children) showed significantly lower overall age-adjusted developmental scores in the exposed children. Delays were seen at all ages and were greater in children who were smaller in size, had neonatal signs of intoxication, and/or had a history of nail deformities. Results of follow-up testing (Stanford-Binet test and Wechsler Intelligence Scale) when the children were 4–7 years old indicate that effects on cognitive development persisted for several years following exposure (Chen et al. 1992). Delays in cognitive development were found in boys at ages 6, 7, 8, or 9 years, with no alterations in girls (Guo et al. 1995). Delays in child development were also observed in children of Yu-Cheng mothers born 7–12 years after the incident; no effects were found in children of Yu-Cheng fathers (Guo et al. 1994b). Li et al. (2015b) found an increased incidence of asymmetrical hearing threshold increases in

Yu-Cheng children tested in early adulthood (see Table 2-8 for additional information). Maternal serum 2,3,4,7,8-pentaCDF levels were associated with increased hearing thresholds (>20 dB) at 250, 500, and 1,000 Hz in the right ear, at 500 and 4,000 Hz in the left ear, and at average hearing thresholds in the right and left ears. An inverse association between hearing loss and maternal serum levels of 2,3,4,7,8-pentaCDF was also found. Decreased measures of distortion product otoacoustic emissions (DPOAE, indicating hearing loss) at 1.5 and 2 Hz in the right ear and at the average threshold levels in both ears were present. Maternal serum 1,2,3,4,7,8-hexaCDF was also associated with an increased hearing threshold (decreased hearing sensitivity at >20 dB) at 4,000 Hz in the left ear, but no other associations were found with this congener.

A general population study found inverse associations between performance on tests for psychomotor development in 6-month-olds and maternal serum 2,3,7,8-tetraCDF, 1,2,3,6,7,8-pentaCDF, and 1,2,3,6,7,8-hexaCDF levels (Nakajima et al. 2006); there were no associations with the mental developmental index scores. In another study by this group (Nakajima et al. 2017), inverse associations between the psychomotor development scores and maternal 2,3,7,8-tetraCDF and 1,2,3,7,8-pentaCDF were observed in males at 6 months, but not in 18-month-olds; an inverse association was found between maternal 1,2,3,4,6,7,8-heptaCDF and mental development index scores in 18-month-old females. Another general population study found associations between neonatal neurological optimality scores and breast milk levels of 2,3,7,8-tetraCDF and 2,3,4,7,8-pentaCDF, but not for other CDF congeners (Huisman et al. 1995). Studies of Vietnamese children in Agent Orange hotspots found associations between breast milk levels of some CDF congeners and performance on tests assessing autism spectrum disorder (Nishijo et al. 2012), eating behaviors (Nguyen et al. 2018), and language skills (Tai et al. 2013). See Table 2-8 for additional information on these studies. In Japan, CDFs were measured in maternal blood samples collected during the third trimester as part of the Sapporo Cohort of the Hokkaido Study on Environment and Children's Health (Yamazaki et al. 2022). A follow-up was done on 55 of the mother-child pairs from the Hokkaido study, and the now school-age children were assessed for event-related brain potentials (ERPs) using a 3-stimulus visual oddball task, which uses visual stimuli and physical responses to measure the P3a and P3b waves. No associations were observed between total CDFs and omission error rate, false alarm rate, or P3a and P3b latency in the difficult test scenario. Reaction time and P3a and P3b amplitude were inversely associated with total CDFs.

A couple of studies evaluated possible effects on the reproductive system of Yu-Cheng children. An increase in the percentage of abnormal sperm morphology was observed in young men exposed *in utero*; there were no alterations in semen volume or sperm count (Guo et al. 2000). Another study of boys found

increased serum estradiol levels at the age of puberty but found no alterations in serum testosterone or follicle stimulating hormone levels at the age of puberty or before puberty (Hsu et al. 2005). In adolescent girls prenatally exposed to Yu-Cheng contaminants, a shorter mean duration of menstrual bleeding per cycle, higher rate of irregular menstrual cycles, and elevated serum estradiol and follicle stimulating hormone levels were found (Yang et al. 2005).

In a study of children living in Agent Orange contaminated areas in Vietnam, inverse associations between maternal breast milk levels of several penta-, hexa-, and heptaCDF congeners and levels of serum dehydroepiandrosterone, androstenedione, and testosterone were found (Oanh et al. 2018); see Table 2-8 for additional information. A similar study found significant decreases in salivary testosterone in girls with increasing maternal concentrations of 2,3,7,8-tetraCDF, 2,3,4,7,8-pentaCDF, 2,3,4,6,7,8-hexaCDF, and 1,2,3,4,6,7,8-heptaCDF, but this was not observed in boys (Pham et al. 2020). Oyama et al. (2021) identified positive associations with a variety of CDFs and progesterone, DHEA, and CYP17 lyase, and inverse associations with testosterone, 3β-HSD, and 17β-HSD, mostly in boys.

Wang et al. (2022) examined the relationship between CDFs in maternal breast milk and steroid hormones in 6-year-old children living near an electronic waste region in China. Inverse associations were observed between 2,3,4,7,8-pentaCDF, 1,2,3,6,7,8-hexaCDF, and total CDFs and DHEA levels, while 1,2,3,7,8-pentaCDF was inversely correlated with androstenedione levels. No associations were observed with testosterone or progesterone, and no differences were found between male and female children. In a study from Denmark, placental levels of several CDFs and thyroid hormones were evaluated in male infants both with and without cryptorchism (Li et al. 2018). The placental concentration of T4 was associated with 1,2,3,4,6,7,8-heptaCDF; T3 was associated with 2,3,7,8-tetraCDF and 1,2,3,7,8-pentaCDF; and rT3 was associated with 1,2,3,7,8-pentaCDF and 2,3,4,6,7,8-hexaCDF. No differences, however, were observed in the placental thyroid hormones between boys born with or without cryptorchidism.

A few studies evaluated potential impairment of the immune system in the children born after the Yu-Cheng incident. Hsu et al. (1985) reported that approximately 20% of hyperpigmented children exposed perinatally died due to pneumonia, bronchitis, and prematurity (Hsu et al. 1985). Lan et al. (1990) reported that the immune status was normal in the children in the Yu-Cheng cohort 7–9 years old. Another study reported increased frequency of parent reported influenza in children born during or after the Yu-Cheng incident (Yu et al. 1998). No alterations in serum IgA, IgG, or IgM levels, leukocyte subpopulations, T-cell markers, B-cell markers, or natural killer cell markers were found. Chao et al.

(1997) reported that children born to the Yu-Cheng cohort had higher incidences of middle ear disease, with the highest incidences occurring in children born closer to the time of the incident. Children with middle ear disease had higher serum levels of 2,3,4,7,8-pentaCDF and 1,2,3,4,7,8-hexaCDF.

2,3,7,8-TetraCDF. The developmental toxicity of 2,3,7,8-tetraCDF was examined in mouse studies that examined a limited number of potential endpoints (Weber et al. 1984, 1985). Hydronephrosis was observed in the offspring of mice administered ≥350 µg/kg on gestation day (GD) 10 and at ≥10 µg/kg on GDs 10–13. Cleft palate was also observed at ≥600 µg/kg on GD10 and at ≥50 µg/kg on GDs 10–13. These effects were observed in the absence of overt signs of maternal toxicity, although increases in maternal relative liver weight were noted. Weber et al. (1984) also reported an increase in fetal mortality at ≥250 µg/kg; this finding was not confirmed in a subsequent study in which mice were administered 300–900 µg/kg on GD 10 (Weber et al. 1985). No developmental effects were observed following single (GD 15) or repeated dose (GDs 8–20) studies in pregnant female SD rats, including fetal viability, sex ratio, fetal body weights, and fetal testis histology (Johnson et al. 2020).

1,2,3,7,8-PentaCDF. One study evaluated the developmental toxicity of 1,2,3,7,8-pentaCDF. No significant alterations in fetal weight or mortality were observed in the offspring of rats administered ≤200 µg/kg on GDs 10–13 (Birnbaum et al. 1987a). An increased incidence of hydronephrosis was observed at ≥30 µg/kg/day on GDs 10–13; cleft palate was reported at ≥100 µg/kg.

2,3,4,7,8-PentaCDF. Several studies evaluated the developmental toxicity of 2,3,4,7,8-pentaCDF in rodents; observed effects included decreases in fetal/offspring body weight, alterations in thymus weight, increases in the incidence of malformations/anomalies, alterations in the liver, reproductive system alterations, and fetal mortality. Decreases in fetal weight were observed in the offspring of rats administered ≥30 µg/kg on GD 8, 10, or 12 (Couture et al. 1989) and in mice administered ≥30 µg/kg/day on GDs 10–13 (Birnbaum et al. 1987a); a second mouse study did not find significant alterations at 30 µg/kg/day on GDs 10–13 (Birnbaum et al. 1987b). In the male and female offspring of rats administered a single dose of 2,3,4,7,8-pentaCDF on GD 15, ED_{50} values of 56.3±15.7 and 140±860 µg/kg, respectively, were estimated for decreases in fetal weights (Taura et al. 2014). This study also found decreases in growth hormone levels in female (ED_{50} of 12.6 µg/kg) and male (ED_{50} of 27.4 µg/kg) fetuses (Taura et al. 2014); the investigators suggested that this may have contributed to the observed growth retardation. Another rat study reported decreases in offspring body weight on postnatal days (PNDs) 10, 15, and 20 in the offspring of rats administered 10 µg/kg on GD 15 and at PND 140 in the 1 and 10 µg/kg groups (Salisbury and Marcinkiewicz 2002).

Most of the available developmental studies on 2,3,4,7,8-pentaCDF examined a limited number of endpoints and exposures were limited to the critical developmental time period for the examined endpoint. A 14% decrease in neonatal thymus weight (14%) was observed in the offspring of rats administered 2 µg/kg on GD 16 (Madsen and Larsen 1989). A cross-fostering experiment in which dams were administered 10 µg/kg 2,3,4,7,8-pentaCDF on GD 16 demonstrated a similar decrease in thymus weight in 1-week-old pups exposed *in utero* and in pups exposed via lactation only (Madsen and Larsen 1989); a greater decrease was observed in pups exposed *in utero* and via lactation. Administration of 80 µg/kg/day on GDs 10–13 resulted in impaired embryonic erythropoiesis in the liver, an increase in the number of hepatocytes, and a reduction in the size and number of sinusoids in the liver, and a narrowed central lobular vein (Khera 1992).

Two studies conducted by Birnbaum et al. (1987a, 1987b) reported increased incidences of hydronephrosis in the offspring of mice administered ≥5 µg/kg/day on GDs 10–13 and cleft palate at ≥30 µg/kg/day. An increase in mortality was noted in the fetuses of rats administered 100 µg/kg on GD 8, 10, or 12 (Couture et al. 1989); there were no alterations in fetal mortality at ≤80 µg/kg/day on GDs 10–13 (Birnbaum et al. 1987a, 1987b).

Impaired development of the reproductive system has been observed in male and female offspring. A decrease in the number of days spent in estrus (approximately 30% decrease) with a concomitant increase in diestrus length was observed in the offspring of rats administered 1 µg/kg on GD 15 (Salisbury and Marcinkiewicz 2002). Decreases in ovulation rate were also observed in the offspring at 1 (60% decrease in the number of ova/rate) and 10 µg/kg (90% decrease). In a second study, decreases in luteinizing hormone levels were observed in the male and female fetuses of rats administered 2,3,4,7,8-pentaCDF on GD 15; the ED_{50} values were 56.3 and 140 µg/kg, respectively (Taura et al. 2014). Taura et al. (2014) also evaluated development and sexual maturation in male offspring of rats administered 2,3,4,7,8-pentaCDF on GD 15. Prolonged mount latency, decreased mount frequency, increased intromission latency, and decreased intromission frequency were reported at 50 µg/kg. The investigators suggested that exposure to 2,3,4,7,8-pentaCDF disrupts testicular steroidogenesis in fetuses due to the reduction in the expression of pituitary luteinizing hormone, which imprints defects in sexual behavior (Taura et al. 2014).

1,2,3,4,7,8-HexaCDF. Increases in mean fetal weights were observed in the offspring of mice administered ≥100 µg/kg 1,2,3,4,7,8-hexaCDF on GDs 10–13 (Birnbaum et al. 1987a); edema was noted

in the fetuses in the 1,000 µg/kg group. There were no significant alterations in fetal mortality at ≤1,000 µg/kg (Birnbaum et al. 1987a, 1987b). An increase in the incidence of hydronephrosis was observed at ≥100 µg/kg (Birnbaum et al. 1987a, 1987b). Cleft palate was also reported at ≥300 µg/kg in the Birnbaum et al. (1987a) study but was not observed at 300 µg/kg in the second study by this group (Birnbaum et al. 1987b).

Mechanisms. It is well documented that orally administered 2,3,7,8-substituted tetra-, penta-, and hexaCDF congeners induce hydronephrosis and cleft palate in mice at doses that are not maternally toxic and that hydronephrosis is induced at lower doses than cleft palate (Birnbaum et al. 1987a, 1987b; Weber et al. 1984, 1985). The kidney and palate were the only tissues examined in mice because studies with 2,3,7,8-TCDD showed that morphogenesis in these tissues is selectively affected (ATSDR 1998). The strain of mouse (C57BL/6N) tested in these oral studies is known to be Ah-responsive (Morrissey and Schwetz 1989). A single intraperitoneal dose of 0.6 mg/kg 2,3,7,8-tetraCDF on GD 12 induced high incidences of cleft palate and hydronephrosis were found in the Ah-responsive inbred mouse strains, but no cleft palates and few fetuses with hydronephrosis were found in the Ah-nonresponsive strains (Hassoun et al. 1984). Ah-nonresponsive mice appear to have a defective Ah receptor (Goldstein and Safe 1989). This evidence and studies of 2,3,7,8-TCDD (ATSDR 1998; Morrissey and Schwetz 1989) indicate that developmental toxicity of CDFs is mediated by the Ah receptor (see Section 2.20). Studies with 2,3,7,8-TCDD indicate that the *in utero* development of hydronephrosis induced by CDFs may be caused by hyperplasia of the ureteral epithelium (Abbot et al. 1987). Both 2,3,4,7,8-pentaCDF and 2,3,7,8-TCDD can cause hemorrhages in placental tissues (embryo-maternal vascular barrier, visceral yolk sac membrane, maternal vascular spaces of the placenta periphery) of mice at teratogenic doses (Khera 1992). It is not known, however, if these hemorrhagic lesions play a role in the induction of cleft palate or hydronephrosis.

Summary. The developmental toxicity of CDFs has been demonstrated in humans and laboratory animals. Perinatal exposure to high levels resulted in skin lesions similar to those observed in adults, decreases in birth weight, delays in cognitive development, and impairment of immune function. A number of effects have been observed in laboratory animals including increases in the prevalence of hydronephrosis and cleft palate, decreases in fetal weight, fetal mortality, and impaired development of the reproductive system. Birnbaum et al. (1987a) compared the potential of 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, and 1,2,3,4,7,8-hexaCDF to induce hydronephrosis and cleft palate. For both endpoints, 2,3,4,7,8-pentaCDF was the most toxic, followed by 1,2,3,4,7,8-hexaCDF. The ED_{50} values

for hydronephrosis were 36, 133, and 342 μg/kg for 2,3,4,7,8-pentaCDF, 1,2,3,7,8-pentaCDF, and 1,2,3,4,7,8-hexaCDF, respectively.

2.18 OTHER NONCANCER

Epidemiological studies evaluated several other noncancer endpoints including tooth and gum alterations, pancreatitis, and metabolic syndrome. Members of the Yusho cohort reported increased prevalences of periodontal disease, gingivitis, pigmentation of the gingiva, and hypersensitivity of teeth (Akahane et al. 2018). Increased prevalence of gum pigmentation was also reported in another study of the Yusho cohort (Imamura et al. 2007) and the Yu-Cheng cohort (Guo et al. 1999). Other noncancer effects examined in only one study of the Yusho cohort included an increased prevalence of pancreatitis (Akahane et al. 2018) and high uric acid levels (Imamura et al. 2009; Matsumoto et al. 2010).

Two studies evaluated the possible association between CDF serum levels and the prevalence of metabolic syndrome (see Table 2-9). Five criteria are used to assess metabolic syndrome: obesity, high blood pressure, high serum triglycerides, low HDL cholesterol levels, and elevated blood glucose. In a study of residents living near a highly dioxin-contaminated site in Taiwan, associations were found between individual CDF congeners, expressed as TEQs, and metabolic syndrome scores (Chang et al. 2010). In the second study of individuals living in Japan, total CDFs TEQ, 2,3,4,7,8-pentaCDF TEQ, and 1,2,3,6,7,8-hexaCDF TEQ serum levels were associated with a higher prevalence of metabolic syndrome (defined as meeting three or more of the five criteria) (Uemura et al. 2009). Both studies reported dose-related trends between CDF congener TEQ levels and metabolic syndrome prevalence.

Table 2-9. Results of Epidemiological Studies Evaluating Exposure to CDFs and Other Noncancer Effects

Reference, study type, and population	Biomarker[a]	Outcome evaluated	Result
Chang et al. 2010 Cross-sectional; 1,490 adults living near a highly dioxin-contaminated site (Taiwan)	Serum 2,3,7,8-tetraCDF TEQ (levels not reported)	Metabolic syndrome[b]	↑ 4th quintile
	Serum 1,2,3,7,8-pentaCDF TEQ (levels not reported)	Metabolic syndrome[b]	↑ 4th quintile
	Serum 2,3,4,7,8-pentaCDF TEQ (levels not reported)	Metabolic syndrome[b]	↑ 2nd quintile
	Serum 1,2,3,4,7,8-hexaCDF TEQ (levels not reported)	Metabolic syndrome[b]	↑ 3rd quintile
	Serum 1,2,3,6,7,8-hexaCDF TEQ (levels not reported)	Metabolic syndrome[b]	↑ 3rd quintile

Table 2-9. Results of Epidemiological Studies Evaluating Exposure to CDFs and Other Noncancer Effects

Reference, study type, and population	Biomarker[a]	Outcome evaluated	Result
	Serum 2,3,4,6,7,8-hexaCDF TEQ (levels not reported)	Metabolic syndrome[b]	↑ 3rd quintile
	Serum 1,2,3,7,8,9-hexaCDF TEQ (levels not reported)	Metabolic syndrome[b]	↔
	Serum 1,2,3,4,6,7,8-heptaCDF TEQ (levels not reported)	Metabolic syndrome[b]	↔
	Serum 1,2,3,4,7,8,9-heptaCDF TEQ (levels not reported)	Metabolic syndrome[b]	↔
	Serum octaCDF TEQ (levels not reported)	Metabolic syndrome[b]	↔
	Serum total CDF TEQ (levels not reported)	Metabolic syndrome[b]	↑ 5th quintile
Uemura et al. 2009 Cross-sectional, 1,374 adults (Japan)	Serum median 2,3,4,7,8-pentaCDF TEQ 3.5 pg/g lipid	Metabolic syndrome[c]	↑ 2nd quintile
	Serum median 1,2,3,6,7,8-hexaCDF TEQ median 0.3 pg/g lipid	Metabolic syndrome[c]	↑ 2nd quintile
	Serum total CDF TEQ 2nd quintile ≥2.90–4.50 pg/g lipid	Metabolic syndrome[c]	↑ 2nd quintile

[a]TEQs were calculated using the WHO 1998 TEF values (van den Berg et al. 1998).
[b]Metabolic syndrome defined at having at least three factors: waist circumference >90 cm in men and 80 cm in women; blood pressure >130/85 mmHg, triglycerides >150 mg/dL; HDL cholesterol levels <40 mg/dL; and fasting blood glucose >100 mg/dL.
[c]Metabolic syndrome defined as having at least three factors: BMI ≥25 kg/m^2; serum triglycerides >150 mg/dL; serum HDL cholesterol levels <40 mg/dL; systolic blood pressure >130 mmHg and/or diastolic blood pressure ≥85 mmHg, or self-reported history of physician-diagnosed hypertension; and HbA1C ≥5.6% or self-reported physician diagnosed diabetes.

↑ = association between biomarker level and outcome; ↓ = inverse association between biomarker level and outcome; ↔ = no association between biomarker level and outcome; BMI = body mass index; CDF = chlorodibenzofuran; HDL = high-density lipoprotein; TEF = toxic equivalency factor; TEQ = toxic equivalency; WHO = World Health Organization

2,3,7,8-TetraCDF. Degranulation of exocrine pancreatic cells were observed in rhesus monkeys dying early after administration of 1,000 µg/kg 2,3,7,8-tetraCDF (Moore et al. 1979).

2,3,4,7,8-PentaCDF. In rats chronically administered ≥0.044 µg/kg 2,3,4,7,8-pentaCDF, squamous hyperplasia of the gingiva was observed (NTP 2006).

2.19 CANCER

Several retrospective mortality studies evaluated the Yusho and Yu-Cheng cohorts and found increases in cancer deaths (Kuratsune et al. 1987; Onozuka et al. 2009, 2020) in the Yusho cohort and no increases in risk of death from all cancers in the Yu-Cheng cohort (Tsai et al. 2007; Yu et al. 1997). A meta-analysis of the Onozuka et al. (2009) Yusho data and unpublished data on the Yu-Cheng cohort reported an association with a pooled SMR of 1.3 (95% CI 1.1–1.6) among male members (Li et al. 2015a); no association was found among females. A 50-year follow-up study of the Yusho cohort found increases in all cancer deaths in males but not in females (Onozuka et al. 2020). An additional assessment determined that males had higher risk of cumulative incidence of cancer-specific mortality than females (Onozuka et al. 2021).

Several studies also examined possible associations for specific tumor sites among the Yusho and Yu-Cheng cohorts, in workers, and in the general population. In the Yusho cohorts, increases in the prevalence of bowel cancer (Akahane et al. 2018), deaths from lung cancer, and deaths from liver cancer (Kuratsune et al. 1987; Onozuka et al. 2009) were observed. The 50-year follow-up study also found increases in lung cancer deaths in males and increases in liver cancer deaths in females (males trended positively but did not reach statistical significance) (Onozuka et al. 2020). In the Yu-Cheng cohort, Li et al. (2013) found increases in the prevalence of neoplasms of the stomach, lymphatic, and hematopoietic tissues among males. The Li et al. (2015a) meta-analysis also examined specific tumor types (cancers of the stomach, rectum, liver, pancreas, lung, female breast, uterus, and leukemia). The only associations found were for lung cancer in males (SMR 1.7, 95% CI 1.2–2.3) and lung cancer for males and females combined (SMR 1.5, 95% CI 1.1–2.1).

In a study of subjects with known exposure to phenoxyacetic acids or potential exposure to CDDs/CDFs, higher levels of 2,3,4,7,8-pentaCDF were found in the seven cases with malignant lymphoproliferative diseases (Hardell et al. 1995). Perrot-Applanat et al. (2021) found no associations between diffuse gastric cancer and the concentration of CDFs in adipose tissue, although increased concentrations of 1,2,3,4,7,8-hexaCDF and 1,2,3,6,7,8-hexaCDF were measured in patients with other cancer types that had metastasized in the peritoneal cavity. Two general population case-control studies found no differences in levels of CDF congeners in breast adipose tissue in breast cancer cases (Hardell et al. 1996) or in abdominal adipose tissue in non-Hodgkin lymphoma cases (Hardell et al. 2001), as compared to controls. A third general population study found no association between breast tissue adipose levels of 2,3,4,7,8-pentaCDF, 1,2,3,6,7,8-hexaCDF, or 1,2,3,4,6,7,8-heptaCDF levels and the risk of breast cancer

(Reynolds et al. 2005). The results of the Hardell et al. (1996, 2001), Perrot-Applanat et al. (2021), and Reynolds et al. (2005) studies are summarized in Table 2-10.

Table 2-10. Results of Epidemiological Studies Evaluating Exposure to CDFs and Cancer Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
Hardell et al. 1996 Case control; 22 patients with invasive breast cancer and 19 controls (Sweden)	Breast tissue median 2,3,7,8-tetraCDF 5.9 pg/g lipid in cases and 4.1 pg/g lipid in controls	Breast cancer	↔
	Breast tissue median 1,2,3,7,8-pentaCDF 1.1 pg/g lipid in cases and 0.6 pg/g lipid in controls	Breast cancer	↔
	Breast tissue median 2,3,4,7,8-pentaCDF 23.8 pg/g lipid in cases and 21.6 pg/g lipid in controls	Breast cancer	↔
	Breast tissue median 1,2,3,4,7,8-hexaCDF 5.3 pg/g lipid in cases and 3.4 pg/g lipid in controls	Breast cancer	↔
	Breast tissue median 1,2,3,6,7,8-hexaCDF 4.2 pg/g lipid in cases and 3.2 pg/g lipid in controls	Breast cancer	↔
	Breast tissue median 1,2,3,7,8,9-hexaCDF 0.1 pg/g lipid in cases and 0.9 pg/g lipid in controls	Breast cancer	↔
	Breast tissue median 1,2,3,4,7,8,9-heptaCDF 0.6 pg/g lipid in cases and 0.7 pg/g lipid in controls	Breast cancer	↔
	Breast tissue median octaCDF 2.3 pg/g lipid in cases and 1.9 pg/g lipid in controls	Breast cancer	↔
Hardell et al. 2001 Case control; 33 patients with non-Hodgkin lymphoma and 39 controls (Sweden)	Abdominal fat mean 2,3,7,8-tetraCDF 0.94 pg/g lipid in cases and 1.5 pg/g lipid in controls	Non-Hodgkin lymphoma	↔
	Abdominal fat mean 1,2,3,7,8-pentaCDF 0.73 pg/g lipid in cases and 0.99 pg/g lipid in controls	Non-Hodgkin lymphoma	↔
	Abdominal fat mean 2,3,4,7,8-pentaCDF 21 pg/g lipid in cases and 21 pg/g lipid in controls	Non-Hodgkin lymphoma	↔

Table 2-10. Results of Epidemiological Studies Evaluating Exposure to CDFs and Cancer Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Abdominal fat mean 1,2,3,4,7,8-hexaCDF 4.0 pg/g lipid in cases and 3.5 pg/g lipid in controls	Non-Hodgkin lymphoma	↔
	Abdominal fat mean 1,2,3,6,7,8-hexaCDF 3.3 pg/g lipid in cases and 3.1 pg/g lipid in controls	Non-Hodgkin lymphoma	↔
	Abdominal fat mean 1,2,3,7,8,9-hexaCDF 0.93 pg/g lipid in cases and 0.97 pg/g lipid in controls	Non-Hodgkin lymphoma	↔
	Abdominal fat mean 1,2,3,4,6,7,8-heptaCDF 9.2 pg/g lipid in cases and 4.3 pg/g lipid in controls	Non-Hodgkin lymphoma	↔
	Abdominal fat mean 1,2,3,4,7,8,9-heptaCDF 0.39 pg/g lipid in cases and 0.53 pg/g lipid in controls	Non-Hodgkin lymphoma	↔
	Abdominal fat mean octaCDF 0.83 pg/g lipid in cases and 0.91 pg/g lipid in controls	Non-Hodgkin lymphoma	↔
Perrot-Applanat et al. 2021 Case control: 14 patients with diffuse gastric cancer, 10 patients with other cancers that had metastasized to the peritoneal cavity, and 8 patients with abdominal surgery for reasons other than cancer (United States)	Adipose tissue median (interquartile range) 2,3,7,8-tetraCDF 0.3 pg/g lipid in controls, 0.3 pg/g lipid in diffuse gastric cancer, and 0.5 pg/g lipid in other cancers	Diffuse gastric cancer	↔
		Other cancer	↔
	Adipose tissue median (interquartile range) 1,2,3,7,8-pentaCDF 0.2 pg/g lipid in controls, 0.1 pg/g lipid in diffuse gastric cancer, and 0.3 pg/g lipid in other cancers	Diffuse gastric cancer	↔
		Other cancer	↔
	Adipose tissue median (interquartile range) 2,3,4,7,8-pentaCDF 9.2 pg/g lipid in controls, 15 pg/g lipid in diffuse gastric cancer, and 12.4 pg/g lipid in other cancers	Diffuse gastric cancer	↔
		Other cancer	↔
	Adipose tissue median (interquartile range) 1,2,3,4,7,8-hexaCDF 1.7 pg/g lipid in controls, 2.9 pg/g lipid in diffuse gastric cancer, and 2.6 pg/g lipid in other cancers	Diffuse gastric cancer	↔
		Other cancer	↑

Table 2-10. Results of Epidemiological Studies Evaluating Exposure to CDFs and Cancer Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Adipose tissue median (interquartile range) 1,2,3,6,7,8-hexaCDF 2.0 pg/g lipid in controls, 3.1 pg/g lipid in diffuse gastric cancer, and 3.2 pg/g lipid in other cancers	Diffuse gastric cancer	↔
		Other cancer	↑
	Adipose tissue median (interquartile range) 1,2,3,7,8,9-hexaCDF 0.1 pg/g lipid in controls, 0.1 pg/g lipid in diffuse gastric cancer, and 0.1 pg/g lipid in other cancers	Diffuse gastric cancer	↔
		Other cancer	↔
	Adipose tissue median (interquartile range) 2,3,4,6,7,8-hexaCDF 0.7 pg/g lipid in controls, 1.1 pg/g lipid in diffuse gastric cancer, and 1.0 pg/g lipid in other cancers	Diffuse gastric cancer	↔
		Other cancer	↔
	Adipose tissue median (interquartile range) 1,2,3,4,6,7,8-heptaCDF 1.1 pg/g lipid in controls, 1.4 pg/g lipid in diffuse gastric cancer, and 1.4 pg/g lipid in other cancers	Diffuse gastric cancer	↔
		Other cancer	↔
	Adipose tissue median (interquartile range) 1,2,3,4,7,8,9-heptaCDF 0.1 pg/g lipid in controls, 0.2 pg/g lipid in diffuse gastric cancer, and 0.1 pg/g lipid in other cancers	Diffuse gastric cancer	↔
		Other cancer	↔
	Adipose tissue median (interquartile range) octaCDF 0.2 pg/g lipid in controls, 0.3 pg/g lipid in diffuse gastric cancer, and 0.3 pg/g lipid in other cancers	Diffuse gastric cancer	↔
		Other cancer	↔
	Adipose tissue median (interquartile range) total CDFs 17.7 pg/g lipid in controls, 23.4 pg/g lipid in diffuse gastric cancer, and 21.4 pg/g lipid in other cancers	Diffuse gastric cancer	↔
		Other cancer	↔
Reynolds et al. 2005 Case control; 79 women with invasive breast cancer and 52 controls with benign breast cancer (United States)	Breast tissue median 2,3,4,7,8-pentaCDF 8 pg/g lipid in cases and 8 pg/g lipid in controls	Invasive breast cancer	↔
	Breast tissue median 1,2,3,4,7,8-hexaCDF 5 pg/g lipid in cases and 4 pg/g lipid in controls	Invasive breast cancer	↔
	Breast tissue median 1,2,3,6,7,8-hexaCDF 4 pg/g lipid in cases and 3 pg/g lipid in controls	Invasive breast cancer	↔

Table 2-10. Results of Epidemiological Studies Evaluating Exposure to CDFs and Cancer Effects

Reference, study type, and population	Biomarker	Outcome evaluated	Result
	Breast tissue median 1,2,3,4,6,7,8-heptaCDF 7 pg/g lipid in cases and 8 pg/g lipid in controls	Invasive breast cancer	↔

↑ = association between biomarker level and outcome; ↓ = inverse association between biomarker level and outcome; ↔ = no association between biomarker level and outcome; CDF = chlorodibenzofuran

2,3,7,8-TetraCDF. In a tumor promotion study using 2,3,7,8-tetraCDF, hairless mice were initiated with a single 5 µmol dermal dose of methylnitronitrosoguanidine (MNNG) in acetone or just acetone followed by twice weekly dermal applications of ≈33.3 µg 2,3,7,8-tetraCDF/kg in acetone for 20 weeks (Poland et al. 1982). Skin papillomas developed in 100% of the mice initiated with MNNG and in 5% of the mice initiated with acetone, compared to 0% in the control group. These findings indicate that 2,3,7,8-tetraCDF had skin tumor promotion activity.

2,3,4,7,8-PentaCDF. The carcinogenic potential of 2,3,4,7,8-pentaCDFs following oral exposure was examined in a study of female rats administered 2,3,4,7,8-pentaCDF via gavage 5 days/week for 2 years (NTP 2006). In rats administered 0.2 µg/kg, increased incidences of hepatocellular adenoma (4/53 compared to 1/53 in controls), cholangiocarcinoma (2/53 compared to 0/53 in controls), and gingival squamous cell carcinoma (3/53 compared to 1/53 in controls) were observed. Although the increases were not statistically significant, NTP considered them to be treatment-related. The investigators noted that these types of neoplasms were also observed in studies of 2,3,7,8-TCDD and PCB 126, which supported the conclusions that the lesions were due to 2,3,4,7,8-pentaCDF exposure. Nonsignificant increases in neoplastic lesions were also observed in the lungs, pancreas, and uterus; some of these lesions were higher than historical controls and the investigators concluded that these lesions may be treatment related. The lesions included cystic keratinizing epithelioma in the lung of one rat in the 0.2 µg/kg group; acinus adenoma or carcinoma in the pancreas in the 0.092 µg/kg group and in the 0.2 µg/kg stop exposure group (administered 2,3,4,7,8-pentaCDF for 30 weeks and allowed to recover for the remainder of the 2-year study); and uterine carcinoma in the 0.092 and 0.2 µg/kg groups. Overall, NTP (2006) considered that the study provided some evidence of the carcinogenicity of 2,3,4,7,8-penta-CDF in female Sprague-Dawley rats. The investigators noted that these types of neoplasms were also observed in studies of 2,3,7,8-TCDD and PCB 126, which supported the conclusions that the lesions were due to 2,3,4,7,8-pentaCDF.

Studies also evaluated the carcinogenicity of 2,3,4,7,8-pentaCDF following dermal exposure. Initiation-promotion studies were performed in which a single 5 µmol dose of MNNG initiator was applied to intact uncovered skin of hairless (hr/hr) mice followed by promotion with twice weekly dermal doses of 0.08–3.3 µg/kg 2,3,4,7,8-pentaCDF for 20 weeks (Hebert et al. 1990). Acetone was used as the vehicle for the MNNG and CDFs. Studies were also conducted in which acetone was used as the control initiator and the mice were exposed to 3.3 µg/kg 2,3,4,7,8-pentaCDF 2 times/week for 20 weeks. There were no significant increases in proliferative lesions of the skin in the mice pretreated with acetone and followed by 2,3,4,7,8-pentaCDF, although there was an observation period following treatment. However, proliferative skin lesions developed in 77.8–94.4% of the mice initiated with MNNG and promoted with ≥0.08 µg/kg 2,3,4,7,8-pentaCDF, compared to 10.5% in the control groups. Most of the lesions were hyperproliferative nodules and squamous cell papillomas.

1,2,3,4,7,8-HexaCDF. The carcinogenicity of 1,2,3,4,7,8-hexaCDF was also evaluated in initiation-promotion studies conducted by Hebert et al. (1990) (see 2,3,4,7,8-pentaCDF section for study details). There were no significant increases in proliferative lesions of the skin in the mice pretreated with acetone and followed by 8.3–33.3 µg/kg 1,2,3,4,7,8-hexaCDF. Proliferative skin lesions developed in 47.1–89.5% of the mice initiated with MNNG and promoted with ≥8.3 µg/kg 1,2,3,4,7,8-hexaCDF, compared to 10.5% in the control groups. Most of the lesions were hyperproliferative nodules and squamous cell papillomas.

Cancer Assessments. IARC concluded that 2,3,4,7,8-pentaCDF is carcinogenic to humans (Group 1) (IARC 2012) and that 2,3,7,8-tetraCDF and 1,2,3,4,7,8-hexaCDF are not classifiable as to their carcinogenicity to humans (Group 3) (IARC 1997); the other congeners have not been evaluated by IARC. HHS (NTP 2021) and EPA (IRIS 2022) have not conducted carcinogenicity assessments.

2.20 MECHANISM OF ACTION

Many CDFs, CDDs, PCBs, and other structurally related halogenated aromatic hydrocarbons are believed to share a common mechanism of action intimately related to similarities in their structural configuration. Most of what is known regarding the mechanism of action of these compounds is based on three main lines of information (i.e., structure-receptor binding relationships, structure-induction relationships, and structure-toxicity relationships) (Goldstein and Safe 1989; Safe 1990a, 1991). Most of the studies providing this information investigated compounds other than CDFs, particularly 2,3,7,8-TCDD and other

CDDs, and used parenteral routes of exposure and/or *in vitro* test systems. The concept of a common mechanism explains why all of these compounds, including CDFs, elicit the same responses and differ only in their relative potency. Most, if not all, of the health effects of CDFs and related compounds are mediated by binding to the Ah receptor, which regulates the synthesis of a variety of proteins via alterations in gene expression. This receptor was identified in the cytosol of mouse liver cells (Poland et al. 1976) and, subsequently, in extrahepatic tissues of laboratory animals, mammalian cell cultures, and human organs and cell cultures. The structure-binding relationships for a series of CDFs were estimated *in vitro* using rat hepatic cytosol preparations (Bandiera et al. 1984b; Mason et al. 1985). Not all CDF congeners showed the same affinity for the Ah receptor; affinity was found to be determined by the chlorine substitution pattern. Those congeners that are isostereomers of 2,3,7,8-TCDD bind with the highest affinity. Tetra- to hexaCDFs that are fully substituted in the lateral two, three, seven, and eight positions are the most active congeners. Affinity constants for CDFs span over range of 4 orders of magnitude, with 2,3,4,7,8-pentaCDF having the highest affinity (EC_{50}=1.5x10^{-8} M, compared to 1.0x10^{-8} M for 2,3,7,8-TCDD). All CDFs tested exhibited saturable binding with the Ah receptor and cooperativity was not a factor in these binding interactions (Farrell et al. 1987). The stereospecific nature of the binding strongly suggests the existence of a biological receptor as a mediator in the responses caused by CDFs.

Structure-toxicity relationships for several CDFs have been studied in immature male Wistar rats *in vivo* and in rat cell cultures *in vitro* (Bandiera et al. 1984b; Holcomb et al. 1988; Mason et al. 1985; Safe et al. 1986). Determination of ED_{50} values for hepatic microsomal AHH induction, inhibition of body weight gain, and thymic atrophy showed that the potencies of CDF congeners were structure-dependent, and that the *in vivo* structure-activity relationships for the toxic endpoints closely matched those observed for their *in vitro* AHH induction potencies (Mason et al. 1985). However, CDF congeners containing vicinal unsubstituted carbon atoms deviated from the linear correlation. A similar CDF congeneric pattern of toxicity was found in splenic response assays in C57BL/6 mice (Davis and Safe 1988; Dickerson et al. 1990) and in thymic atrophy and liver hypertrophy in male Wistar rats (Yoshihara et al. 1981). These results, along with results obtained with other halogenated aromatic hydrocarbons (summarized in Safe 1990a), are consistent with, and provide support for, the common receptor-mediated mechanism of action.

CDFs, as well as the other related halogenated aromatic hydrocarbons, induce a variety of microsomal enzyme activities such as cytochrome P-450IAl-dependent monooxygenases primarily in the liver. The most widely studied of these responses are induction of hepatic AHH and EROD both in mammalian cell cultures and in laboratory rodents (Bandiera et al. 1984b; Brewster et al. 1988; DeVito et al. 1993;

Goldstein and Safe 1989; Goldstein et al. 1978; Holcomb et al. 1988; Kawano and Hiraga 1978; Mason et al. 1985; Nebert et al. 1975; Safe 1990a; Safe et al. 1986). Results from a study in male Wistar rats in which the inductive potency of 13 CDF congeners was tested following intraperitoneal dosing showed that only those congeners substituted in carbon positions 2, 3, 7, and 8 exhibited typical 3-methyl-cholanthrene (MC)-type induction (Yoshihara et al. 1981). Those congeners having two or less chlorine substitutions in the lateral positions did not induce EROD activity. Results from a similar study showed that the structure-activity relationships for liver enzyme inductive potency of a series of CDFs were comparable to those reported for the structure-binding relationships (Mason et al. 1985). Furthermore, a linear correlation was observed between AHH induction *in vitro* and *in vivo*, providing further support to a common receptor-mediated mechanism of action for CDFs.

The dioxin-like compounds bound to the cytosolic Ah receptor translocate to the cell nucleus and dimerize with the Ah receptor nuclear translocator (ARNT) protein (Denison et al. 2011; Safe 2001; Zeytun et al. 2002). The Ah receptor-ARNT heterodimer binds with dioxin responsive elements (DREs) which are specific DNA recognition sites (Denison et al. 2011; Safe 2001). The array of genes that can be affected is large with diverse functions (Sutter and Greenlee 1992; Zeytun et al. 2002). The most extensively studied is cytochrome P450 1A1 (CYP1A1) (Kurachi et al. 2002; Sutter and Greenlee 1992; Whitlock 1999). Studies with 2,3,7,8-TCDD have identified other gene products that are induced or repressed. Over 290 genes in the liver can be altered by 2,3,7,8-TCDD (Boutros et al. 2009); these genes are associated with responses to chemical stress/xenobiotics (e.g., CYP1A1, glutathione S-transferase, glucose-6-phosphate dehydrogenase), lipid and cholesterol metabolism (e.g., fatty acid binding protein, lipase, fatty acid synthase, retinol binding protein), nitrogen and amino acid metabolism (e.g., aspartate aminotransferase, alanine aminotransferase, ornithine transcarbamylase), carbohydrate metabolism (e.g., glucokinase, glucose-6-phosphate transfer protein, pyruvate carboxylase), bile acid synthesis, and bile transport (Boverhof et al. 2006; Fletcher et al. 2005; Kurachi et al. 2002). Ah receptor target genes are located in a number of tissues other than the liver including the kidneys, thymus, and spleen (Boutros et al. 2009; Zeytun et al. 2002). Ultimately, newly synthesized enzymes and macromolecules resulting from the pleiotropic response to the CDF-receptor complex are responsible for many of the effects caused by CDFs and other halogenated aromatic hydrocarbons.

Studies with 2,3,7,8-TCDD provide *in vitro* evidence of a nongenomic mechanisms involving the Ah receptor, but not requiring ARNT (Matsumura 2009). These mechanisms appear to contribute to the inflammatory response via cytosolic phospholipase A2 (cPLA2), Cox-2, Src kinase, and other protein kinases and phosphatases.

Oxidative stress is another proposed mechanism for the toxicity of CDFs. Significant increases in the production of superoxide anion and lipid peroxidation were identified in the liver and brain tissues of rats administered 2,3,4,7,8-pentaCDF 5 days/week for 13 weeks (Hassoun et al. 2000) or 30 weeks (Hassoun et al. 2002). The increases in the biomarkers of oxidative stress were dose-related. The responses in the brain and liver were similar. In the 30-week study, increases in lipid peroxidation plateaued between 0.02 and 0.092 µg/kg and increased again at the highest dose (0.2 µg/kg) (Hassoun et al. 2002).

2.21 GENOTOXICITY

A small number of *in vitro* and *in vivo* studies have evaluated the genotoxicity of CDFs. The mutagenicity of several CDF congeners was evaluated in microorganisms; the results are summarized in Table 2-11. In assays with several strains of *Salmonella typhimurium* bacteria, octaCDF and 2,3,7,8-tetraCDF were not mutagenic with or without metabolic activation (Schoeny 1982). In assays with the yeast, *Saccharomyces cerevisiae*, without exogenous metabolic activation, 2,3,7,8-tetraCDF did not induce forward mutations or inter- or intragenic recombinations (Fahrig et al. 1978).

Table 2-11. Genotoxicity of Chlorodibenzofurans (CDFs) *In Vitro*

			Results		
			Activation		
Species (test system)	CDF congener	Endpoint	With	Without	Reference
Salmonella typhimurium (TA98, TA100, TA1535, TA1537, TA1978)	2,3,7,8-tetraCDF	Gene mutation	–	–	Schoeny 1982
Saccharomyces cerevisiae	2,3,7,8-tetraCDF	Forward mutations	NT	–	Fahrig et al. 1978
S. cerevisiae	2,3,7,8-tetraCDF	Recombinations	NT	–	Fahrig et al. 1978
Human peripheral lymphocytes	1,2,4,7,8-pentaCDF	Sister chromatid exchange	–	–	Lundgren et al. 1988
Human peripheral lymphocytes	1,2,4,7,8-pentaCDF	Chromosome aberrations	–	–	Lundgren et al. 1988
Human peripheral lymphocytes	2,3,4,6,7-pentaCDF	Sister chromatid exchange	–	–	Lundgren et al. 1988
Human peripheral lymphocytes	2,3,4,6,7-pentaCDF	Chromosome aberrations	–	–	Lundgren et al. 1988
Human peripheral lymphocytes	2,3,4,7,8-pentaCDF	Sister chromatid exchange	–	–	Lundgren et al. 1988

Table 2-11. Genotoxicity of Chlorodibenzofurans (CDFs) *In Vitro*

Species (test system)	CDF congener	Endpoint	Results		Reference
			Activation		
			With	Without	
Human peripheral lymphocytes	2,3,4,7,8-pentaCDF	Chromosome aberrations	–	–	Lundgren et al. 1988
Human peripheral lymphocytes	1,2,3,4,7,8-hexaCDF	Sister chromatid exchange	–	–	Lundgren et al. 1988
Human peripheral lymphocytes	1,2,3,4,7,8-hexaCDF	Chromosome aberrations	–	–	Lundgren et al. 1988
S. typhimurium (TA98, TA100, TA1535, TA1537, TA1978, TA92, TS24, TA2322, TA2637)	octaCDF	Gene mutation	–	–	Schoeny 1982

[a]Results were only positive in assays conducted by one of three laboratories.
[b]Results were positive when assay was conducted in a desiccator; results were negative when tested in standard assay.

+ = positive results; (+) = weakly positive results; – = negative results; NT = not tested

Limited information was located regarding genotoxic effects of CDFs in humans or animals. The levels of sister chromatid exchanges and chromosome aberrations were examined in peripheral lymphocytes of 35 Yu-Cheng women nonsmokers 5 years after they consumed the contaminated rice oil (Lundgren et al. 1988). As compared to a control group of 24 women nonsmokers, no significant alterations in frequencies of sister chromatid exchange or chromosomal aberrations in lymphocytes were observed. Oxidative stress, which may have resulted from DNA-single strand breaks, was examined in hepatic and brain tissues in rats administered via gavage 2,3,4,7,8-pentaCDF 5 days/week for 13 weeks (Hassoun et al. 2000). There was a significant dose-related increase in DNA single-strand breaks in hepatic and brain tissues after exposure to 2,3,4,7,8-pentaCDF (Hassoun et al. 2000).