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 of the toxicology of chlorinated dibenzofurans (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

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

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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 first by route of exposure-inhalation, oral, and dermal; and then by health effect-death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods-acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

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

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user’s perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of CDFs are indicated in Table 2.2.

CDFs are a class of structurally similar chlorinated hydrocarbons containing two benzene rings fused to a central furan ring (see chemical structure Section 3.1). Based on the number of chlorine substituents (one to eight) on the benzene rings, there are eight homologues of CDFs (monochlorinated through octachlorinated). Each homologous group contains one or more isomers. There are 135 possible CDF isomers, including 4 monochlorinated dibenzofurans (monoCDFs), 16 dichlorinated dibenzofurans (diCDFs), 28 trichlorinated dibenzofurans (triCDFs), 38 tetrachlorinated dibenzofurans (tetraCDFs), 28 pentachlorinated dibenzofurans (pentaCDFs), 16 hexachlorinated dibenzofurans (hexaCDFs), 4 heptachlorinated dibenzofurans (heptaCDFs) and 1 octachlorinated dibenzofuran (octaCDF). The term congener is used to refer to any one particular isomer. Mono-, di-, and trichlorinated CDFs are not considered in this profile.

Health effects have been evaluated in humans exposed to undefined mixtures of congeners of CDFs. Information regarding health effects in animals exposed to CDFs was located for the following congeners: 1,2,4,6,7,9-heptachlorodibenzofuran (1,2,4,6,7,9-heptaCDF); 1,2,3,4,6,8,9-heptachlorodibenzofuran (1,2,3,4,6,8,9-heptaCDF); 1,2,4,6,7,9-hexachlorodibenzofuran (1,2,4,6,7,9-hexaCDF); 1,2,3,4,7,8-hexachlorodibenzofuran (1,2,3,4,7,8-hexaCDF); 1,2,3,6,7,8-hexachlorodibenzofuran (1,2,3,6,7,8-hexaCDF); 1,2,3,4,6,7,8,9-octachlorodibenzofuran (1,2,3,4,6,7,8,9-

octaCDF); 2,3,4,7&pentachlorodibenzofuran (2,3,4,7-pentaCDF); 1,2,3,7,8-pentachlorodibenzofuran (1,2,3,7,8-pentaCDF); 1,2,3,4,8-pentachlorodibenzofuran (1,2,3,4,8-pentaCDF), 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-tetraCDF). Some of the animal studies used mixtures of isomers which are described in appropriate sections of the profile. Of all the CDF congeners, those containing chlorine in the 2,3,7,8 carbon positions, particularly 2,3,7,8-tetraCDF and 2,3,4,7,8-pentaCDF, have been most extensively studied in animals.

CDDs frequently occur with CDFs in the environment. Because of this and due to evidence of a common mechanism of action, total toxicity of a CDFKDD mixture involves both CDFs and CDDs. CDDs appear to usually, but not always, contribute more to total toxicity than CDFs. CDDs are evaluated in a separate ATSDR toxicological profile (ATSDR 1994).

2.2.1 Inhalation Exposure

No studies were located regarding the following health effects in humans or animals after inhalation exposure to CDFs:

2.2.1 .I Death

2.2.1.2 Systemic Effects

2.2.1.3 Immunological Effects

2.2.1.4 Neurological Effects

2.2.1.5 Reproductive Effects

2.2.1.6 Developmental Effects

2.2.1.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

No studies were located regarding cancer in humans or animals after inhalation exposure to CDFs.

2.2.2 Oral Exposure

Much of the information that pertains to human health effects of CDFs comes from large numbers of people who consumed rice oil contaminated with PCBs 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 PCBs were 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 ≈l0 months, and Yu-Cheng involved at least 2,061 victims exposed over ≈9 months (Chen et al. 1985b; 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 the interactive effects of CDFs and PCBs and other components of the contaminated rice oils 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 comparisons with Japanese workers with higher PCB blood levels who had few or none of the symptoms present in the rice oil poisonings, decreasing serum levels of PCBs in victims with persisting health effects, 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 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 1990; 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. 1985b). 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. 1985b). 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 are 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).

2.2.2.1 Death

There was no significant increase in the number of deaths from nonmalignancies or all causes in 887 male or 874 female Yusho victims (Kuratsune et al. 1987). As discussed in Section 2.2.2.8, some increased mortality from malignant neoplasms was observed. Twenty-four deaths were observed in 2,061 cases of Yu-Cheng poisoning identified by the end of 1983 (Hsu et al. 1985). The number of expected deaths was not reported, but half of the deaths were attributed to nonmalignant or malignant liver disease (see Section 2.2.2.2). No more recent comprehensive data on Yu-Cheng deaths are available (Rogan 1989). Deaths in infants born to mothers with Yusho and Yu-Cheng exposure are discussed in Section 2.2.2.6.

Information on lethality of CDFs in animals following acute oral exposure is available for 2,3,7,8-tetraCDF and 2,3,4,7,8-pentaCDF administered by gavage. Due to a long latent period for the onset of toxicity, reliable determination of toxic dose requires a sufficient observation period (typically 30 days in rodents). An LD_{50} of 916 μg/kg has been estimated for 2,3,4,7,8-pentaCDF in male Fischer-344 rats (Brewster et al. 1988). A CDF mixture containing 88% 2,3,7,8-tetraCDF (remainder primarily an unidentified pentaCDF) did not cause death in C57Bl/6Fh mice when tested at doses ≤6,000 μg/kg (Moore et al. 1976). Single 2,3,7,8-tetraCDF doses of 1,000 μg/kg and higher, but not 500 μg/kg, were lethal in rhesus monkeys observed for 60 days, but small numbers of animals (two to four) were tested (Moore et al. 1979). The Hartley guinea pig is the most sensitive of the species tested as indicated by lethality following single doses of 2,3,7,8-tetraCDF or 2,3,4,7,8-pentaCDF as low as 10 μg/kg (Ioannou et al. 1983; Moore et al. 1976, 1979).

Intermediate duration studies have evaluated the lethality of 2,3,7,8-tetraCDF and various pentaCDF congeners in animals. 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 (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). Dietary administration of 10 pg 2,3,4,7,8-pentaCDF/kg/day for 13 weeks caused >90% mortality in 1va:SIV 50 (SD) rats (Pluess et al. 1988a; Poiger et al. 1989). This CDF was more toxic than the 1,2,3,7,8-penta-, 1,2,3,4,8-pentaCDF and 1,2,3,6,7,-hexaCDF congeners, which caused no deaths when similarly administered at dosages of ≤10, ≤300, and ≤10 μg/kg/day, respectively (Pleuss et al. 1988; Poiger et al. 1989).

No studies were located regarding lethality in animals after chronic oral exposure to CDFs.

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 end points. Single and repeated doses were extremely toxic, causing death at levels as low as 10 μg/kg and 0.2-l μg/kg/day, respectively. A wasting syndrome was the major toxic effect at lethal doses in most species (see Section 2.2.2.2), but this may not be the only cause of death.

The LD_{50} value and reliable LOAEL values for death in each species and acute- and intermediate-Duration categories for each congener are recorded in Table 2- 1 and plotted in Figure 2- 1.

2.2.2.2 Systemic Effects

The highest NOAEL values and all reliable representative LOAEL values for each systemic effect in each species and acute- and intermediate-duration categories for each congener tested are recorded in Table 2- 1 and plotted in Figure 2-l.

Respiratory Effects. Clinical observations strongly suggest that Yusho and Yu-Cheng patients experienced frequent or more severe respiratory infections (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). Information on immune status in Yusho and Yu-Cheng patients is discussed in Section 2.2.2.3.

TABLE 2-1. Levels of Significant Exposure to CDFs - Oral

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (µg/kg/day)	LOAEL (effect) Less serious (µg/kg/day)	LOAEL (effect) Serious (µg/kg/day)	Reference	Congener
ACUTE EXPOSURE									
Death									
1	Rat	(GO)	1 d 1x/d				916 (LD50)	Brewster et al. 1988	penta$_1$
2	Gn pig	(GO)	1 d 1x/d				10 (100% mortality)	Moore et al. 1979	penta$_1$
3	Gn pig	(GO)	1 d 1x/d				10 (100% mortality)	Moore et al. 1979	tetra
4	Monkey	(GO)	1 d 1x/d				1000 (50% mortality)	Moore et al. 1979	tetra
Systemic									
5	Rat	(GO)	1 d 1x/d	Resp	2000			Brewster et al. 1988	penta$_1$
				Cardio		500 (nail hemorrhages)			
				Gastro	250	500 (epithelial hyperplasia of nonglandular stomach)			
				Hemato		100 (decreased hemoglobin, MCHC, MCV)			
				Hepatic		100 (lipid accumulation, increased serum cholesterol)			
				Renal	1000	2000 (64% increased BUN in moribund animals, 34% increased relative kidney weight)			
				Other	250	500 (17% body weight loss)			

TABLE 2-1. Levels of Significant Exposure to CDFs - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (µg/kg/day)	LOAEL (effect) Less serious (µg/kg/day)	LOAEL (effect) Serious (µg/kg/day)	Reference	Congener
6	Rat	(GO)	1 d 1x/d	Hepatic	40			Doyle and Fries 1986	$hexa_1$
7	Rat	(GO)	1 d 1x/d	Hepatic	40			Doyle and Fries 1986	octa
8	Rat	(GO)	1 d 1x/d	Hepatic	40			Doyle and Fries 1986	tetra
9	Rat	(GO)	1 d 1x/d	Hepatic Other (body weight)	53 53			Ahlborg et al. 1989	$penta_1$
10	Rat	(GO)	1 d 1x/d	Hepatic	40			Doyle and Fries 1986	$hepta_1$ $hepta_2$
11	Rat	(GO)	1 d 1x/d	Hepatic	40			Doyle and Fries 1986	$penta_1$
12	Gn pig	(GO)	1 d 1x/d	Resp Cardio Gastro Hemato Musc/skel Hepatic Renal Derm/oc Other	15 15 15 15 1 15 15	5 (reduced muscle mass) 10 (epithelial hyperplasia of kidney, ureter and bladder)	10 (50% body weight loss, adrenal hemorrhage)	Moore et al. 1979	tetra

TABLE 2-1. Levels of Significant Exposure to CDFs - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (µg/kg/day)	LOAEL (effect) Less serious (µg/kg/day)	LOAEL (effect) Serious (µg/kg/day)	Reference	Congener
13	Gn pig	(GO)	1 d 1x/d	Resp	30			Moore et al. 1979	penta$_1$
				Cardio	30				
				Gastro	30				
				Hemato	30				
				Musc/skel	1	3 (reduced muscle mass)			
				Hepatic	30				
				Renal		10 (epithelial hyperplasia of kidney, ureter and bladder)			
				Derm/oc	30				
				Other			10 (adrenal hemorrhage)		
14	Mouse	(GO)	1 d 1x/d	Resp	6000			Moore et al. 1976, 1979	tetra
				Cardio	6000				
				Gastro	6000				
				Musc/skel	6000				
				Renal	6000				
				Derm/oc	6000				
15	Monkey	(GO)	1 d 1x/d	Gastro	500		1000 (hemorrhage and ulcers)	Moore et al. 1979	tetra
				Hemato			500 (anemia, lymphopenia, neutrophilia)		
				Hepatic	500	1000 (increased SGOT, gall bladder and bile duct hypertrophy)			
				Renal	500	1000 (increased BUN)			
				Derm/oc			500 (facial edema, occluded or dilated meibomian, ceruminous and sebaceous glands, eyelash and nail loss, epidermal hyperkeratosis)		
				Other			500 (moderate to severe body fat loss)		

TABLE 2-1. Levels of Significant Exposure to CDFs - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (µg/kg/day)	LOAEL (effect) Less serious (µg/kg/day)	LOAEL (effect) Serious (µg/kg/day)	Reference	Congener
Immunological									
16	Rat	(GO)	1 d 1x/d			100 (30% decreased thymus weight)	500 (thymic atrophy)	Brewster et al. 1988	$penta_1$
17	Gn pig	(GO)	1 d 1x/d			3[b] (mild thymic lymphoid hypoplasia)	10 (thymic atrophy)	Moore et al. 1979	$penta_1$
18	Gn pig	(GO)	1 d 1x/d			5 (mild thymic lymphoid hypo-plasia)	10 (thymic atrophy)	Moore et al. 1979	tetra
19	Mouse	(GO)	once			208 (ED50 for decreased antibody response to SRBC)		Kerkvliet et al. 1985	$hepta_3$
20	Mouse	(GO)	1 d 1x/d		6000			Moore et al. 1979	tetra
21	Monkey	(GO)	1 d 1x/d				1000 (thymic atrophy)	Moore et al. 1979	tetra
Developmental									
22	Rat	(GO)	1 d Gd8,10 or 12 1x/d			30 (decreased fetal body weight)	100 (increased fetal mortality)	Couture et al. 1989	$penta_1$
23	Rat	(GO)	Gd16 1x/d		0.5	2 (14% decreased relative neonatal thymus weight)		Madsen and Larsen 1989	$penta_1$
24	Mouse	(GO)	4 d Gd10-13 1x/d				100 (hydronephrosis)	Birnbaum et al. 1987b	$hexa_2$
25	Mouse	(GO)	4 d Gd10-13 1x/d				5 (hydronephrosis)	Birnbaum et al. 1987b	$penta_1$

TABLE 2-1. Levels of Significant Exposure to CDFs - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (µg/kg/day)	LOAEL (effect) Less serious (µg/kg/day)	LOAEL (effect) Serious (µg/kg/day)	Reference	Congener
26	Mouse	(GO)	1 d Gd10 1x/d				250 (fetal mortality, hydronephrosis)	Weber et al. 1984	tetra
27	Mouse	(GO)	4 d Gd10-13 1x/d				10 (hydronephrosis)	Weber et al. 1984	tetra
28	Mouse	(GO)	4 d Gd10-13 1x/d		10		30 (hydronephrosis)	Birnbaum et al. 1987a	$penta_2$
29	Mouse	(GO)	4 d Gd10-13 1x/d		3		10 (hydronephrosis)	Birnbaum et al. 1987a	$penta_1$
30	Mouse	(GO)	4 d Gd10-13 1x/d				100 (hydronephrosis, cleft palate)	Birnbaum et al. 1987a	$hexa_2$
Reproductive									
31	Mouse	(GO)	4 d Gd 10-13 1x/d				80 (hemorhagic lesions in placenta)	Khera 1992	$penta_1$
32	Rat	(GO)	1 d 1x/d		2000			Brewster et al. 1988	$penta_1$
33	Gn pig	(GO)	1 d 1x/d			5 (hypocellularity of seminiferous tubules)		Moore et al. 1979	tetra
34	Gn pig	(GO)	1 d 1x/d			3 (hypocellularity of seminiferous tubules)		Moore et al. 1979	$penta_1$
INTERMEDIATE EXPOSURE									
Death									
35	Rat	(F)	13 wk				10 (92% mortality)	Pluess et al. 1988a; Poiger et al. 1989	$penta_1$

TABLE 2-1. Levels of Significant Exposure to CDFs - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (µg/kg/day)	LOAEL (effect) Less serious (µg/kg/day)	LOAEL (effect) Serious (µg/kg/day)	Reference	Congener
36	Gn pig	(GO)	6 wk 1d/wk 1x/d				1 (30% mortality)	Luster et al. 1979a, 1979b	tetra
37	Monkey	(F)	2 mo				2.1 (33% mortality)	McNulty et al. 1981	tetra
38	Monkey	(F)	6 mo				0.21 (33% mortality)	McNulty et al. 1981	tetra
Systemic									
39	Rat	(F)	4 wk	Hemato			50 (hemolytic anemia)	Oishi and Hiraga 1978	$mixture_1$
				Hepatic		50 (porphyria)			
40	Rat	(F)	13 wk	Cardio	300			Pluess et al. 1988b; Poiger et al. 1989	$penta_3$
				Hemato	300				
				Hepatic	300				
				Renal	300				
				Other	300				
41	Rat	(F)	13 wk	Cardio	10			Pluess et al. 1988a; Poiger et al. 1989	$penta_1$
				Hemato	10				
				Hepatic		0.1[c] (increased serum bilirubin, decreased serum triglycerides)			
				Renal	10				
				Other	0.1	1 (11% decreased body weight gain)	10 (47-54% body weight loss)		
42	Rat	(F)	13 wk	Cardio	10			Pluess et al. 1988b; Poiger et al. 1989	$penta_2$
				Hemato	10				
				Hepatic	1	10 (increased liver weight, vacuolization with lipid accumulation, single cell necrosis)			
				Renal	10				
				Other	1	10 (6.5-11% decreased body weight)			

TABLE 2-1. Levels of Significant Exposure to CDFs - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (µg/kg/day)	LOAEL (effect) Less serious (µg/kg/day)	LOAEL (effect) Serious (µg/kg/day)	Reference	Congener
43	Rat	(F)	13 wk	Cardio	10			Pluess et al. 1988b; Poiger et al. 1989	$hexa_3$
				Hemato	10				
				Hepatic	0.1	1 (increase liver weight, vacuolization with lipid accumulation, single cell necrosis)			
				Renal	10				
				Other	1	10 (14-20% decreased body weight gain)			
44	Rat	(F)	4 wk	Cardio	960			Oishi et al. 1978	$mixture_1$
				Hemato		97 (decreased hemoglobin, hematocrit and MCV, increased MCHC)			
				Hepatic		97 (increased liver weight and lipid content)			
				Renal	960				
				Derm/oc	97		960 (chloracne)		
				Other		97 (15% decreased body weight gain)			
45	Gn pig	(GO)	6 wk 1d/wk 1x/d	Hemato	1			Luster et al. 1979a, 1979b	tetra
				Other	1				
46	Mouse	(GO)	30 d 22 doses 1x/d	Hemato			300 (37% decreased total leukocytes)	Moore et al. 1979	tetra
				Hepatic		30 (25% increased relative liver weight)			
				Other (body weight, clinical signs)	300				

TABLE 2-1. Levels of Significant Exposure to CDFs - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (µg/kg/day)	LOAEL (effect) Less serious (µg/kg/day)	LOAEL (effect) Serious (µg/kg/day)	Reference	Congener
47	Monkey	(F)	2 mo	Gastro		2.1 (intramucosal cysts)		McNulty et al. 1981	tetra
				Hemato	2.1				
				Hepatic		2.1 (altered bile duct epithelium)			
				Derm/oc			2.1 (periorbital edema, facial and body hair and nail loss, absent sebaceous glands)		
48	Monkey	(F)	6 mo	Gastro		0.21 (mucosal metaplasia)		McNulty et al. 1981	tetra
				Hemato	0.21				
				Hepatic		0.21 (altered bile duct epithelium)			
				Derm/oc			0.21 (periorbital edema, meibomian gland enlargement, partial sebaceous gland atrophy, *hyperkeratotic* nail beds)		
Immunological									
49	Rat	(F)	13 wk		300			Pluess et al. 1988b; Poiger et al. 1989	$penta_3$
50	Rat	(F)	13 wk		0.1	1 (decreased thymus weight)	10 (thymic atrophy)	Pluess et al. 1988b; Poiger et al. 1989	$hexa_3$
51	Rat	(F)	13 wk			0.1 (decreased thymus weight)	1 (thymic atrophy)	Pluess et al. 1988a; Poiger et al. 1989	$penta_1$
52	Rat	(F)	13 wk		1	10 (decreased thymus weight)		Pluess et al. 1988b; Poiger et al. 1989	$penta_2$

TABLE 2-1. Levels of Significant Exposure to CDFs - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (µg/kg/day)	LOAEL (effect) Less serious (µg/kg/day)	LOAEL (effect) Serious (µg/kg/day)	Reference	Congener
53	Rat	(F)	4 wk			97 (decreased thymus weight)		Oishi et al. 1978	$mixture_1$
54	Gn pig	(GO)	6 wk 1d/wk 1x/d		0.17		0.5 (thymic atrophy, macrophage inhibition)	Luster et al. 1979a, 1979b	tetra
55	Mouse	(GO)	4 wk 1d/wk 1x/d		10	100 (decreased thymus weight)		Oishi and Hiraga 1980	$mixture_2$
56	Mouse	(GO)	30 d 22 doses 1x/d			300 (17% decreased thymus weight)		Moore et al. 1979	tetra
57	Monkey	(F)	6 mo				0.21 (thymic atrophy)	McNulty et al. 1981	tetra
58	Monkey	(F)	2 mo				2.1 (thymic atrophy)	McNulty et al. 1981	tetra
Reproductive									
59	Rat	(F)	13 wk		10			Pluess et al. 1988b; Poiger et al. 1989	$hexa_3$
60	Rat	(F)	4 wk		97	960 (decreased relative seminal vesicle weight and testosterone concentration in testes)		Oishi et al. 1978	$mixture_1$
61	Rat	(F)	13 wk		10			Pluess et al. 1988b; Poiger et al. 1989	$penta_2$
62	Rat	(F)	13 wk		300			Pluess et al. 1988b; Poiger et al. 1989	$penta_3$

TABLE 2-1. Levels of Significant Exposure to CDFs - Oral (continued)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (µg/kg/day)	LOAEL (effect) Less serious (µg/kg/day)	LOAEL (effect) Serious (µg/kg/day)	Reference	Congener
63	Rat	(F)	13 wk		10			Pluess et al. 1988a; Poiger et al. 1989	$penta_1$

[a]The number corresponds to entries in Figure 2-1.
[b]Used to derive an acute oral Minimal Risk Level (MRL) of 0.001 µg/kg/day for 2,3,4,7,8-pentaCDF; dose divided by an uncertainty factor of 1,000 (10 for extrapolation from animals to humans, 10 for human variability, 10 for use of a LOAEL) and by a modifying factor of 3 to adjust for lack of neurological studies in animals.
[c]Used to derive an intermediate oral MRL of 0.00003 µg/kg/day for 2,3,4,7,8-pentaCDF; dose divided by an uncertainty factor of 1,000 (10 for extrapolation from animals to humans, 10 for human variability, 10 for use of a LOAEL) and by a modifying factor of 3 to adjust for lack of neurological studies in animals.

BUN = blood urea nitrogen; Cardio = cardiovascular; CDFs = chlorinated dibenzofurans; d = day(s); Derm/oc = dermal/ocular; (F) = feed; Gastro = gastrointestinal; Gd = gestation day; Gn pig = guinea pig; (GO) = gavage-oil; Hemato = hematological; LD50 = lethal dose; 50% kill; LOAEL = lowest-observed-adverse-effect level; MCHC = mean corpuscular hemoglobin concentrations; MCV = mean corpuscular volume; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; SGOT = serum oxaloacetic transaminase; wk = week(s); x = times

$hepta_1$ = 1,2,3,4,6,7,9-heptachlorodibenzofuran
$hepta_2$ = 1,2,3,4,6,8,9-heptachlorodibenzofuran
$hepta_3$ = 1,2,3,4,6,7,8-heptachlorodibenzofuran
$hexa_1$ = 1,2,4,6,7,9-hexachlorodibenzofuran
$hexa_2$ = 1,2,3,4,7,8-hexachlorodibenzofuran
$hexa_3$ = 1,2,3,6,7,8-hexachlorodibenzofuran
$mixture_1$ = synthesized mixture containing 2 tetraCDFs, 4 pentaCDFs, and 4 hexaCDFs (specific congeners not reported but average chlorine number is 4.7)
$mixture_2$ = CDF mixture containing 88% pentaCDFs and 12% tetraCDFs (specific congeners not reported)
octa = 1,2,3,4,6,7,8,9-octachlorodibenzofuran
$penta_1$ = 2,3,4,7,8-pentachlorodibenzofuran
$penta_2$ = 1,2,3,7,8-pentachlorodibenzofuran
$penta_3$ = 1,2,3,4,8-pentachlorodibenzofuran
tetra = 2,3,7,8-tetrachlorodibenzofuran

FIGURE 2-1. Levels of Significant Exposure to CDFs - Oral

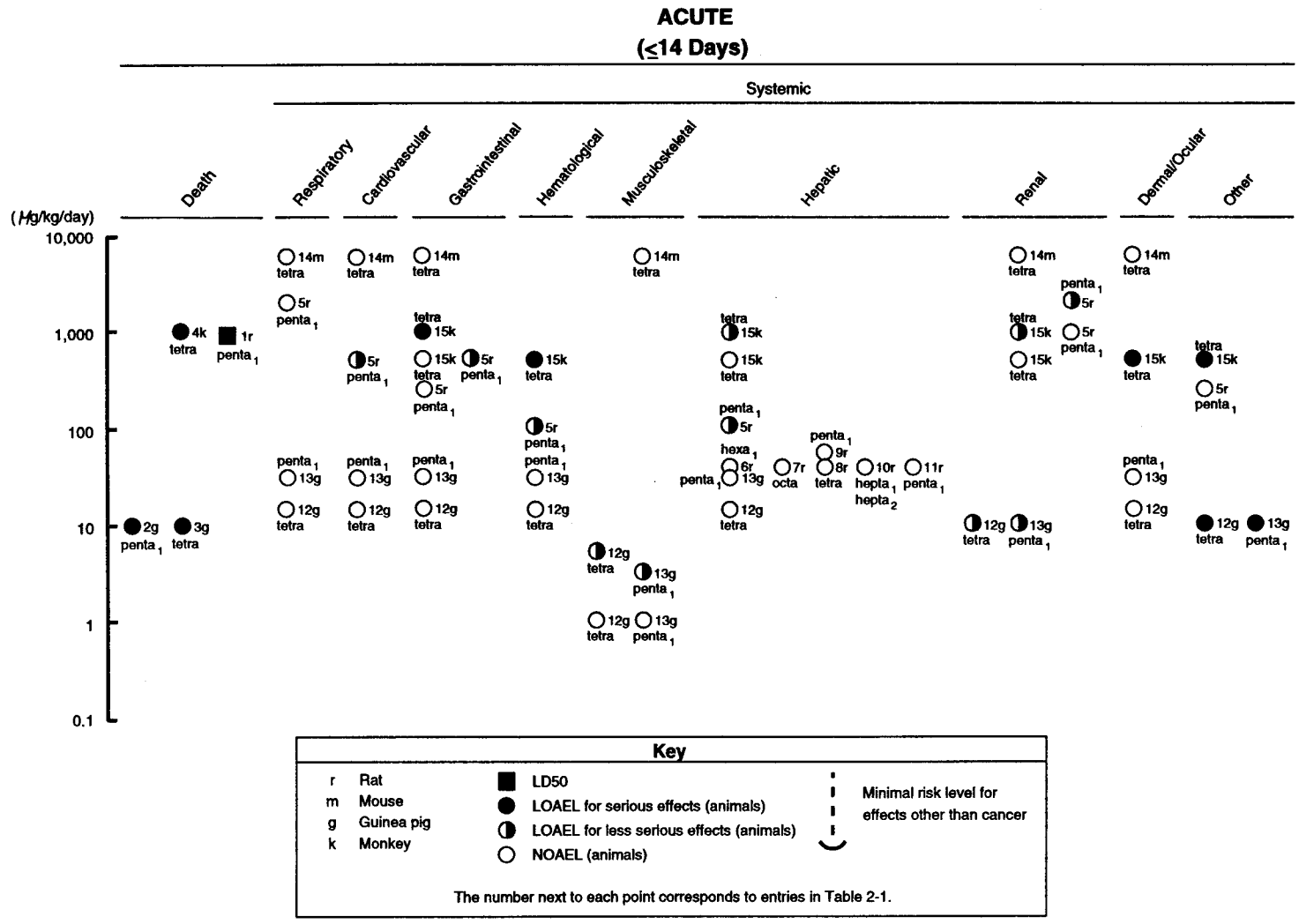


FIGURE 2-1. Levels of Significant Exposure to CDFs - Oral *(continued)*

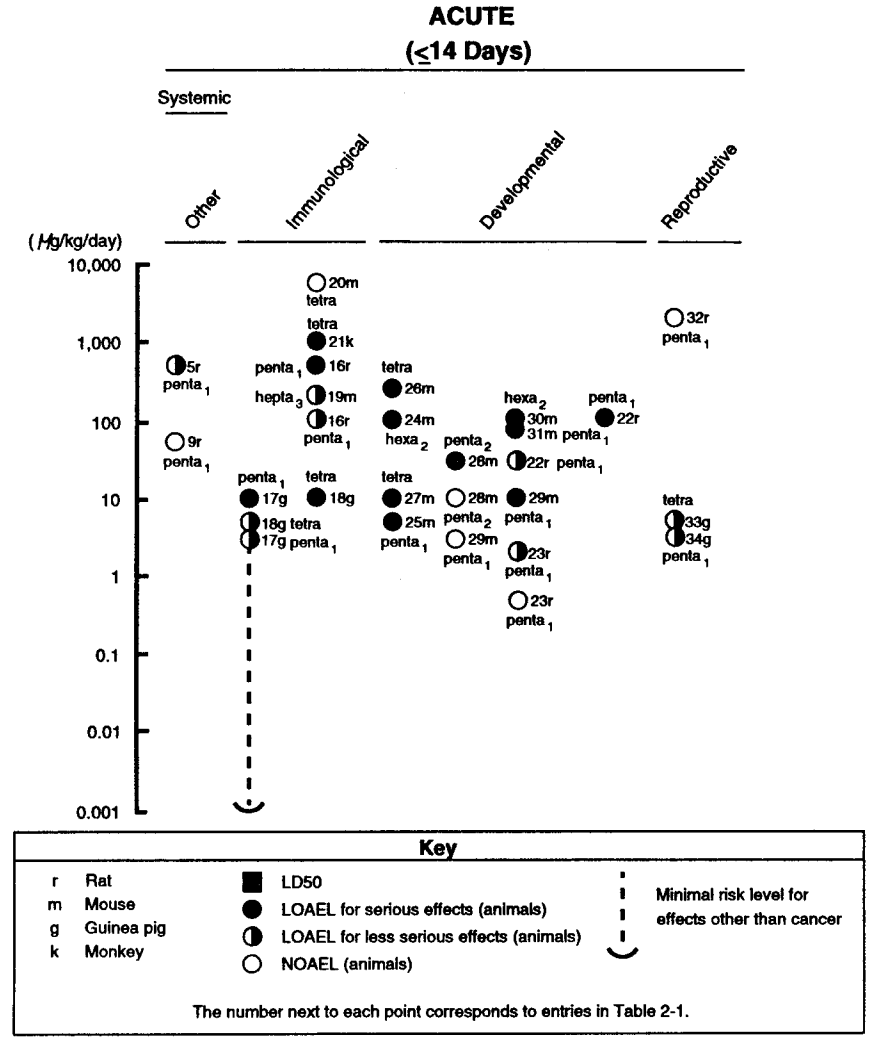


FIGURE 2-1. Levels of Significant Exposure to CDFs - Oral *(continued)*

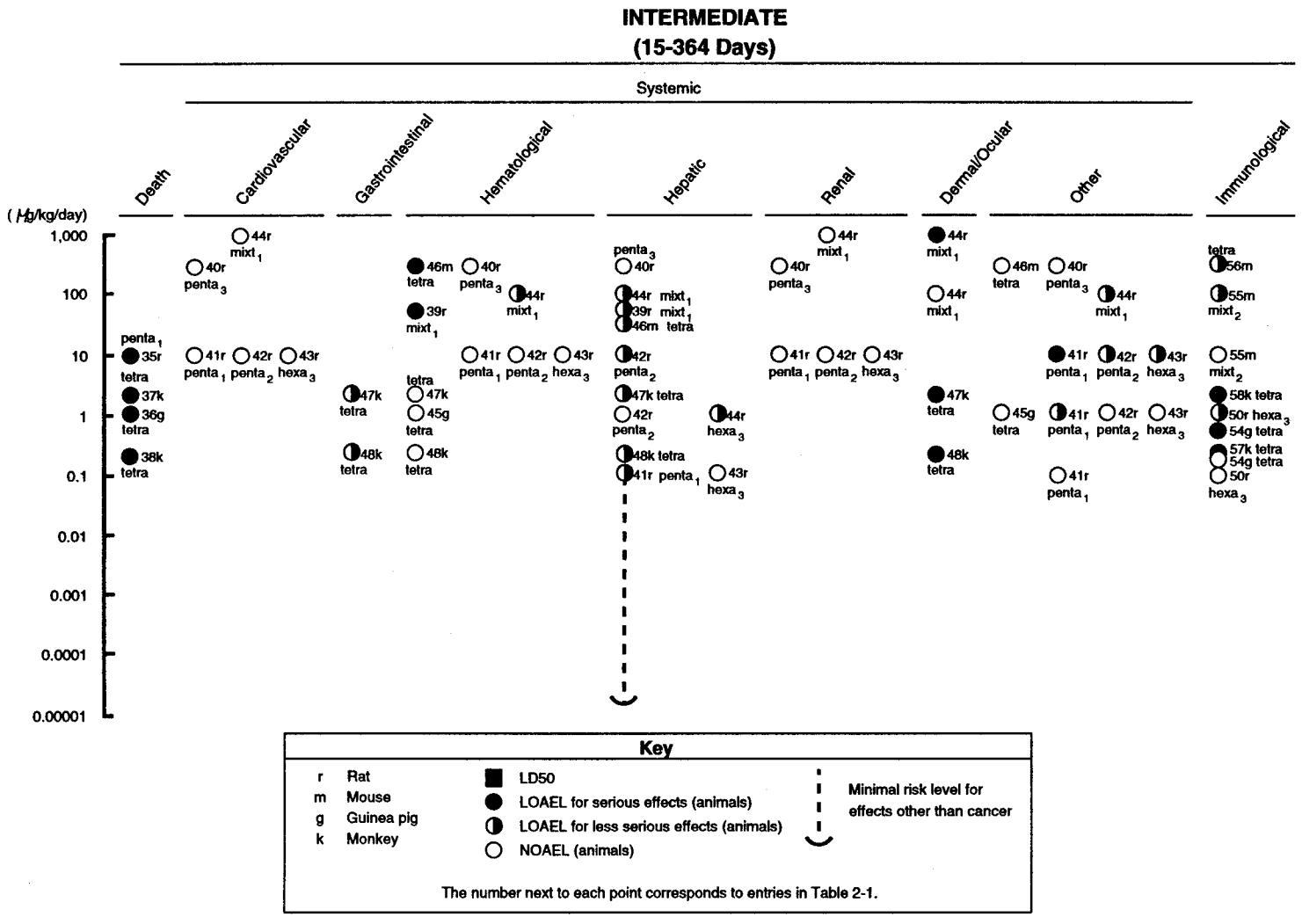
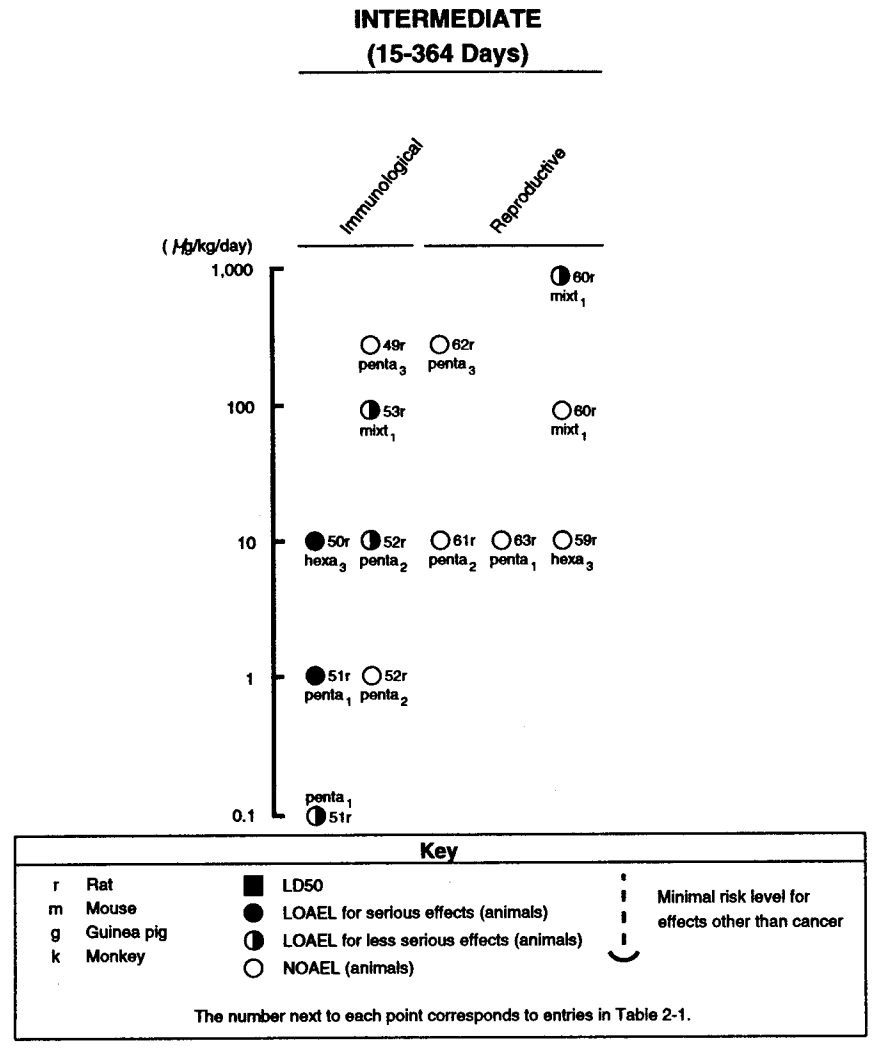


FIGURE 2-1. Levels of Significant Exposure to CDFs - Oral *(continued)*



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No histological alterations were observed in the trachea or lungs of Hartley guinea pigs that were administered a single, nonlethal, gavage dose of ≤5 μg/kg 2,3,7,8-tetraCDF or ≤3 μg/kg 2,3,4,7,8-pentaCDF, in guinea pigs that were similarly treated with single, lethal doses ≤15 ug/kg 2,3,7,8-tetraCDF or 30 μg/kg 2,3,4,7,8-pentaCDF, or in C57B1/6Fh mice that were similarly treated with nonlethal doses ≤6,000 μg/kg 2,3,7,8-tetraCDF (Moore et al. 1979). Fischer-344 rats that were administered a lethal gavage dose of 2,000 μg/kg 2,3,4,7,8-pentaCDF showed no pulmonary histological changes (Brewster et al. 1988). The animals that received the nonlethal doses were examined after 30 days of observation.

No studies were located regarding respiratory effects in animals after intermediate or chronic duration oral exposure to CDFs.

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, but longer term studies have not been performed, nonhuman primates were not tested, and only two congeners were evaluated (2,3,7,8-tetraCDF and 2,3,4,7,8-pentaCDF).

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after oral exposure to CDFs.

No histological alterations were observed in the heart of Hartley guinea pigs that were administered a single nonlethal gavage dose of ≤5 μg/kg 2,3,7,8-tetraCDF or ≤3 μg/kg 2,3,4,7,8-pentaCDF, in guinea pigs that were similarly treated with single, lethal doses ≤15 μg/kg 2,3,7,8-tetraCDF or 30 μg/kg 2,3,4,7,8-pentaCDF, or in C57B1/6Fh mice that were similarly treated with nonlethal doses of ≤6,000 μg/kg 2,3,7,8-tetraCDF (Moore et al. 1979). The animals that received nonlethal doses were examined after 30 days of observation. Hemorrhages under the nails were observed in 4 of 13 Fischer-344 rats that died following a single, lethal gavage dose of 2,3,4,7,8-pentaCDF (Brewster et al. 1988), but it is unclear if the lowest lethal dose (500 μg/kg) is the LOAEL for this effect. Hemorrhages also were observed in the stomach of monkeys and adrenal of guinea pigs given lethal oral doses of 2,3,7,8-tetraCDF and/or 2,3,4,7,8-pentaCDF (Moore et al. 1979) (see Gastrointestinal Effects and Other Systemic Effects).

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No histological alterations were observed in the heart of 1va:SIV 50 (SD) rats that were administered dietary dosages of ≤10 μg/kg/day 2,3,4,7,8-pentaCDF, 1,2,3,7,8-pentaCDF or 1,2,3,6,7,8-hexaCDF, or ≤300 μg/kg/day 1,2,3,4,8-pentaCDF, for 13 weeks (Pluess et al. 1988a, 1988b; Poiger et al. 1989). 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 Sprague-Dawley rats, but histology was not evaluated (Oishi et al. 1978). The increased relative heart weight is likely due to concurrent lower body weight (see Other System Effects). No studies were located regarding cardiovascular effects in animals after chronic duration oral exposure to CDFs.

The animal studies with 2,3,7,8-tetraCDF and 2,3,4,7,8-pentaCDF provide some evidence that CDFs can induce hemorrhagic effects at acute lethal doses, but studies of these and other 2,3,7,8-substituted CDF congeners give no indication of altered cardiac histology after acute or intermediate duration exposure. No differences are apparent among the rodent species tested and effects of CDFs on cardiac function have not been evaluated.

Gastrointestinal Effects. Early symptoms in 89 male and 100 female Yusho patients included vomiting (23.6% and 28% frequencies) and diarrhea (19.1% and 17%) (Kuratsune 1989). Additional information on possible gastrointestinal effect of CDFs in humans was not located.

No histological alterations were observed in the esophagus, stomach, or intestine of Hartley guinea pigs that were administered a single, nonlethal, gavage dose of ≤5 μg/kg 2,3,7,8-tetraCDF or ≤3 μg/kg 2,3,4,7,8-pentaCDF, in guinea pigs that were similarly treated with single lethal doses ≤15 μg/kg 2,3,7,8-tetraCDF or 30 μg/kg 2,3,4,7,8-pentaCDF, or in C57B1/6Fh mice that were similarly treated with nonlethal doses of ≤6,000 μg/kg 2,3,7,8-tetraCDF (Moore et al. 1979). The animals that received nonlethal doses were examined after 30 days of observation. In contrast, epithelial hyperplasia of the nonglandular stomach, characterized by acanthosis and hyperkeratosis, was observed in Fischer-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 250 μg/kg and lower doses (Brewster et al. 1988). Similarly, gastric lesions developed in rhesus monkeys that were administered a single 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 small intestine occurred in three of six monkeys.

Gastric mucosal changes occurred in rhesus monkeys treated with dietary 2,3,7-tetraCDF in intermediate duration studies (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 considered to be compound-related, No studies were located regarding gastrointestinal effects in animals after chronic duration oral exposure to CDFs.

The animal studies indicate that the gastric mucosa is a target of CDFs in monkeys and rats at nearlethal and lethal doses 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.

Hematological Effects. Mild normocytic anemia and leukocytosis are fairly consistent findings in Yu-Cheng patients (Rogan 1989).

Various hematological alterations have been observed in animals treated with 2,3,78-substituted CDF congeners, but decreased hemoglobin appears to be the only consistent finding. In acute duration studies, Fischer-344 rats that were administered single gavage doses of ≥100 μg/kg 2,3,4,78-pentaCDF and evaluated 7-21 days following treatment showed dose-related decreased hemoglobin concentration, mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) (Brewster et al. 1988). 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. Single gavage doses of ≤30 μg/kg 2,3,4,7,8-pentaCDF or ≤15 μg/kg 2,3,7,8-tetraCDF produced no treatment-related hematological changes in Hartley guinea pigs observed for 30 days (Moore et al. 1979). Mild anemia, mild lymphopenia, and marked neutrophilia developed in rhesus monkeys following single ≥500 μg/kg doses of 2,3,7,8-tetraCDF (Moore et al. 1979).

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In intermediate duration studies, C57BL/6Fh mice treated with 300 μg/kg/day 2,3,7,8-tetraCDF by gavage on 22 days in a 30-day period had decreased total leukocytes with no changes in differential count or other hematological indices (Moore et al. 1979). The NOAEL in this study is not known because lower dosages were not evaluated. Other studies with 2,3,7,8-tetraCDF showed normal leukocyte counts (other indices not evaluated) in guinea pigs treated by weekly gavage with ≤l μg/kg for 6 weeks (Luster et al. 1979a, 1979b), and no alterations in blood cell counts in rhesus monkeys treated by diet with 0.21 μg/kg/day for 6 months or 2.1 μg/kg/day for 2 months (McNulty et al. 1981). Although peripheral blood cell counts were normal in these monkeys, histological examinations showed hypocellularity of the bone marrow. Hematological evaluations were performed in 1va:SIV 50 (SD) rats that were fed 2,3,4,7,8-pentaCDF, 1,2,3,4,8-pentaCDF, 1,2,3,7,8-pentaCDF or 1,2,3,6,7,8-hexaCDF for 13 weeks (Pluess et al. 1988a, 1988b; Poiger et al. 1989). A few alterations (e.g., decreased hemoglobin, decreased thrombocyte count, increased platelets, increased white blood cells and/or increased packed cell volume) were observed at dosages of ≥0.1 μg/kg/day 2,3,4,7,8-pentaCDF and 10 μg/kg/day 1,2,3,7,8-pentaCDF or 1,2,3,6,7,8-hexaCDF, but the only consistent finding was 7-9% decreased hemoglobin. Due to the uncertain physiological significance of the small percentage decreases in hemoglobin with no changes in red blood cell count, and the sporadic occurrence of other hematological effects which could be related to general systemic toxicity, none of the changes are considered to be adverse. No treatment-related hematologic alterations occurred in the rats treated with 1,2,3,4,8-pentaCDF (the only non-2,3,7,8-substituted congener tested) at dosages as high as 300 μg/kg/day. 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).

The above findings indicate that mild anemia is a fairly consistent hematological effect of 2,3,7,8-substituted CDFs in humans and animals. Responses, however, varied among congener, animal species, and dose and duration of exposure.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after oral exposure to CDFs.

Reduced muscle mass, but no histological alterations in muscle, was observed in Hartley guinea pigs that were administered a single gavage dose of ≥5 μg/kg 2,3,7,8-tetraCDF or ≥3 μg/kg

2,3,4,7,8-pentaCDF (Moore et al. 1979). The reduced muscle mass appears to be a manifestation of a generalized wasting syndrome (see Other Systemic Effects).

No studies were located regarding musculoskeletal effects in animals after intermediate or chronic duration oral exposure to CDFs.

Hepatic Effects. Mild hepatic alterations have been described in Yusho and Yu-Cheng patients (Kuratsune 1989; Rogan 1989). Markedly increased serum triglycerides with unchanged serum cholesterol was an abnormal laboratory finding peculiar to both Yusho and Yu-Cheng exposure (Okumura et al. 1979; Uzawa et al. 1969). The elevated triglycerides generally persisted for several years following exposure and subsequently declined to normal. Yusho patients have shown few abnormalities in serum levels of liver enzymes or in liver function tests (Kuratsune 1989), but elevations in serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) are fairly consistent findings in Yu-Cheng patients (Rogan 1989). Increased urinary excretion of uroporphyrin, but not coproporphyrin or porphobilinogen, is another consistent finding in Yu-Cheng patients, including children born to exposed mothers (Chang et al. 1980; Gladen et al. 1988; Lu et al. 1980).

Ultrastructural changes, particularly alterations in the endoplasmic reticulum, and pleomorphic and enlarged mitochondria appear to be the predominant morphological finding in Yusho patients (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 et al. 1989).

Hepatic effects in animals following acute duration oral exposure to CDFs were mild to moderate in severity. Microsomal mixed function oxygenase (MFO) enzyme induction is one of the most sensitive hepatic effects of CDFs and is consistent with ultrastructural changes in the endoplasmic reticulum. This effect was not considered adverse if it occurred with no pathologic or other biochemical changes. Assays for activity of the MFO aryl hydrocarbon hydroxylase (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 (Doyle and Fries 1986). Hepatic AHH activity was significantly increased (2.1- to

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 1,2,3,4,6,7,8,9-octaCDF), and the 2,7- and 2,8-diCDFs, but other doses and end points were not evaluated. A single gavage dose of 53 μg/kg 2,3,4,7,8-pentaCDF produced hepatic biochemical changes (increased microsomal 7-ethoxyresorufin 0-deethylase [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). Single gavage doses of ≥100 μg/kg 2,3,4,7,8-pentaCDF were hepatotoxic to Fischer-344 rats as indicated by a spectrum of dose-related effects observed after 35 days, including increased EROD activity and relative liver weight, increased serum cholesterol (nearly doubled in all groups 7 days postexposure), and lipid accumulation in liver with biliary hyperplasia at ≥500 μg/kg (Brewster et al. 1988). Effects including increased SGOT activity and gall bladder and bile duct epithelial hypertrophy, but no other changes in liver histology or relative weight, were observed in rhesus monkeys 60 days after single lethal doses (≥1,000 μg/kg) of 2,3,7,8-tetraCDF (Moore et al. 1979). Studies with Hartley guinea pigs showed no histological alterations in the liver or gall bladder 30 days after single gavage doses as high as 15 μg/kg 2,3,7,8-tetraCDF or 30 μg/kg 2,3,4,7,8-pentaCDF, which were lethal (Moore et al. 1979). Unspecified hepatic histological alterations suggestive of mild liver toxicity were observed in C57BV6Fh mice examined 30 days after a single nonlethal dose of 6,000 μg/kg/day 2,3,7,8-tetraCDF (Moore et al. 1976, 1979).

Intermediate duration studies indicate that 2,3,7,8-substituted CDFs are more hepatotoxic than other congeners. Liver toxicity was assessed in Iva:SIV 50 (SD) rats that were fed 2,3,4,7,8-pentaCDF, 1,2,3,4,8-pentaCDF, 1,2,3,7,8-pentaCDF, or 1,2,3,6,7,8-hexaCDF for 13 weeks (Pluess et al. 1988a, 1988b; Poiger et al. 1989). A spectrum of effects including increased relative liver weight, increased serum alkaline phosphatase, cholesterol and bilirubin, decreased serum triglycerides and SGPT, and/or fatty and necrotic changes, were observed at dosages of ≥0.1 μg/kg/day 2,3,4,7,8-pentaCDF, ≥1 μg/kg/day 1,2,3,6,7,8-hexaCDF and 10 μg/kg 1,2,3,7,8-pentaCDF. No treatment-related hepatic alterations occurred in the rats treated with 1,2,3,4,8-pentaCDF at dosages as high as 300 μg/kg/day. 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). Based on the LOAEL for hepatic effects (increased serum bilirubin, decreased serum triglycerides) in rats, an intermediate oral MRL of 0.00003 ug/kg/day was calculated for 2,3,4,7,8-pentaCDF as described in footnote “c” in Table 2-l. Increased height and number of

goblet cells in the bile duct epithelium were the only hepatic histological alterations found in rhesus monkeys that died from dietary ingestion of 2,3,7,8-tetraCDF dosages of 0.21 μg/kg/day for 6 months or 2.1 μg/kg/day for 2 months (one animal per dose examined) (McNulty et al. 1981). These alterations were not accompanied by chemical-related changes in serum levels of liver-associated enzymes. Relative liver weight was increased in C57BL/6Fh mice 30 days following gavage of ≥30 μg/kg/day 2,3,7,8-tetraCDF on 22 days in a 30-day period, but lower dosages were not tested and other hepatic end points were not reported (Moore et al. 1979).

No studies were located regarding hepatic effects in animals after chronic duration oral exposure to CDFs.

The previous studies indicate that the liver is an important target of CDFs. Animal tests performed primarily in rats and monkeys indicate that congeners substituted in the 2,3,7,8 positions are most hepatotoxic. Insufficient studies are available on other species to assess differences in sensitivity to CDFs.

Renal Effects. No studies were located regarding renal effects in humans after oral exposure to CDFs.

Acute duration studies have 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 Hartley guinea pigs 30 days after single gavage doses of ≥10 μg/kg 2,3,7,8tetraCDF or 2,3,4,7,8-pentaCDF (Moore et al. 1979). It is unclear from this report whether or not this effect also occurred in guinea pigs treated with 5 μg/kg 2,3,7,8-tetraCDF or 3 μg/kg 2,3,4,7,8-pentaCDF, the only nonlethal dose groups in which histology was evaluated. Increased relative kidney weight, decreased absolute kidney weight, and 64% increased blood urea nitrogen (BUN) was found in Fischer-344 rats observed for 35 days following a single gavage dose 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 2000 mg/kg, this dose is a LOAEL. No histological alterations were observed in the kidneys of C57B1/6Fh 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 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).

In intermediate duration studies, there were no treatment-related kidney histological alterations in 1va:SIV 50 (SD) rats that ingested ≤10 μg/kg 2,3,4,7,8-pentaCDF, 1,2,3,7,8-pentaCDF or 1,2,3,6,7,8-hexaCDF, or ≤300 μg/kg 1,2,3,4,8-pentaCDF, via diet for 13 weeks (Pluess et al. 1988a, 1988b; Poiger et al. 1989). 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. No studies were located regarding renal effects in animals after chronic duration exposure.

In conclusion, 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. Information on other congeners and species is not available.

Derma/Ocular Effects. Effects in the skin and eyes, the most obvious manifestations of Yusho and Yu-Cheng exposure, have been observed in the majority of cases and have been evaluated in numerous studies (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, comedo formation, acneform 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. Most patients 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. Improvement of the ocular changes was gradual and occurred with improvement of dermal effects.

Limited information is available on dermal or ocular effects of CDFs in animals following acute oral exposure. Rhesus monkeys that were treated with single, nonlethal (500 μg/kg) or lethal

(≥1000 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 eyelashes and fingernails, exudate with occlusion and squamous metaplasia of eyelid (meibomian) and ear canal (ceruminous) glands, epidermal hyperkeratosis, and dilation of sebaceous gland ducts, and, at 1,500 ug/kg, follicular hyperkeratosis. No skin or eye histological alterations were observed in Hartley guinea pigs 30 days after single gavage doses of ≤15 μg/kg 2,3,7,8-tetraCDF or ≤30 μg/kg 2,3,4,7,8-pentaCDF, or in C57BU6Fh mice similarly treated with 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 periorbital edema, meibomian gland enlargement, 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 eyelid reddening and thickening and partial facial hair loss after 1 month, and body hair and nail loss and absent sebaceous glands. Surviving monkeys were completely recovered 2-3 months after either exposure. Chloracne-like lesions developed on the ear of Sprague-Dawley 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). No studies were located regarding dermal effects in animals after chronic duration oral exposure to CDFs.

As discussed above, effects in the skin and eye are the most obvious manifestations of CDF toxicity on humans and animals. The studies in animals, although limited by number of congeners and species tested, indicate that 2,3,7,8-tetraCDF and 2,3,4,7,8-pentaCDF are active and that monkeys are more sensitive than rodents.

Other Systemic Effects. One of the major effects of CDFs in animals is a wasting syndrome that has been observed in acute and intermediate duration studies (no chronic studies have been performed). The syndrome is characterized by progressive decreased weight gain, with immediate moderate to severe body weight loss generally occurring at near-lethal doses. Single gavage doses have caused wasting effects in guinea pigs at ≥3 μg/kg 2,3,4,7,8-pentaCDF or ≥5 μg/kg 2,3,4,7,8-pentaCDF or 2,3,7,8-tetraCDF (Moore et al. 1976, 1979), rats at ≥500 μg/kg 2,3,4,7,8-pentaCDF (Brewster et al. 1988) and monkeys at ≥500 μg/kg 2,3,7,8-tetraCDF (Moore et al. 1979). In intermediate duration studies, body weight gain was decreased in Iva:SIV 50 (SD) rats fed

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≥1 μg/kg/day dosages of 2,3,4,7,8-pentaCDF or a 10 μg/kg/day dosage of 1,2,3,7,8-pentaCDF or 1,2,3,6,7&hexaCDF for 13 weeks, with a higher lethal dosage of 2,3,4,7,8-pentaCDF (10 μg/kg/day) causing approximately 50% weight loss (Pluess et al. 1988a, 1988b; Poiger et al. 1989). Similar treatment with ≤300 μg/kg/day 1,2,3,4,8-pentaCDF did not affect rat body weight. Body weight gain was decreased in Sprague-Dawley rats exposed to ≥97 μg/kg/day dietary dosages of an uncharacterized mixture of two tetra-, four penta-, and four hexaCDFs for 4 weeks (Oishi et al. 1978). Studies with a CDF mixture similar to that in Yusho oil showed decreased body weight gain in Sprague-Dawley rats exposed to 44 μg/kg/day for 22 days and body weight loss in cynomolgus monkeys exposed to 8 μg/kg/day for 80-113 days (Kunita et al. 1984). Rhesus monkeys administered 0.21 μg/kg/day 2,3,7,8-tetraCDF in diet for 6 months had normal growth except for a brief period of rapid weight loss prior to death (McNulty et al. 1981). Other gavage studies of 2,3,7,8-tetraCDF showed no treatment-related effects on body weight in Hartley guinea pigs administered weekly doses of ≤1 μg/kg/day for 6 weeks (Luster et al. 1979a, 1979b) or in C57BL/6Fh(J67) mice treated with ≤300 μg/kg/day on 22 days in a 30-day period (Moore et al. 1979). In conclusion, animals studies have demonstrated that 2,3,7,8-substituted tetra-, penta-, and hexaCDF congeners induce wasting in all species and duration categories tested.

Endocrinological evaluations of Yu-Cheng patients found a tendency for increased urinary excretion of 17-ketosteroids and 17-hydroxycorticosteroids (Nagai et al. 1971). Effects on reproductive endocrinology in Yu-Cheng patients have also been reported (see Section 2.2.2.5)

Limited information is available on effects of CDFs on endocrine organs in animals. Adrenal hemorrhage, but no histological changes in the thyroid or pancreas, were found in Hartley guinea pigs that received single, lethal, gavage doses of ≥10 μg/kg/day 2,3,7,8-tetraCDF or 2,3,4,7,8-pentaCDF (Moore et al. 1979). Adrenal histology was normal in Iva:SIV 50 (SD) rats administered dietary dosages of ≤10 μg/kg/day 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, or 1,2,3,6,7,8-hexaCDF, or ≤300 μg/kg/day 1,2,3,4,8-pentaCDF, for 13 weeks (Pluess et al. 1988a, 1988b; Poiger et al. 1989). These dosages were sublethal except for 10 μg/kg/day 2,3,4,7,8-pentaCDF. 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). Effects on the thymus are discussed in Section 2.2.2.3 (Immunological Effects).

2.2.2.3 Immunological Effects

Clinical observations strongly suggest that Yusho and Yu-Cheng patients experienced frequent or more severe skin and respiratory infections and lowered resistance to illness (Kuratsune 1989; Rogan 1989). Various changes in immune status have been reported in Yusho and Yu-Cheng patients, including decreased serum IgA and IgM levels and lymphocyte subpopulations, diminished phagocyte complement and IgG receptors, and diminished delayed-type skin hypersensitive response (Chang et al. 1981, 1982a, 1982b; Lu and Wu 1985; Nakanishi et al. 1985; Shigematsu et al. 1971). Immune status was normal in children 7-9 years old who had *in utero* Yu-Cheng exposure (Lan et al. 1990).

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% have been observed. Decreased thymus weight and histologic atrophic changes in thymus (e.g., lymphoid depletion) occurred following single gavage doses of ≥100 μg/kg/day 2,3,4,7,8-pentaCDF in Fischer-344 rats (Brewster et al. 1988), ≥3 μg/kg/day 2,3,4,7,8-pentaCDF and ≥5 μg/kg/day 2,3,7,8-tetraCDF in Hartley guinea pigs (Moore et al. 1979), and ≥1,000 μg/kg/day 2,3,7,8-tetraCDF in rhesus monkeys (Moore et al. 1979). Based on the LOAEL for thymic histopathology in guinea pigs, an acute oral MRL of 0.001 μg/kg/day was calculated for 2,3,4,7,8-pentaCDF as described in footnote "b" in Table 2-l. Thymus weight was also decreased in Sprague-Dawley rats fed ≈44 μg/kg/day of a CDF mixture similar to that in Yusho oil for 10 days (Kunita et al. 1984). No thymic or splenic histological alterations were observed in C57b1/6Fh mice 30 days after a single gavage dose of 6,000 μg/kg 2,3,7,8-tetraCDF (Moore et al. 1979). In intermediate duration studies with Iva:SIV 50 (SD) rats, dose-related changes progressing from decreased thymus weight to thymic atrophy resulted from 13-week dietary treatment with ≥0.1 μg/kg/day 2,3,4,7,8-pentaCDF, ≥1 μg/kg/day 1,2,3,6,7,8-hexaCDF or ≥10 μg/kg/day 1,2,3,7,8-pentaCDF, but not with ≤300 mg/kg/day 1,2,3,4,8-pentaCDF (Pluess et al. 1988a, 1988b; Poiger et al. 1989). 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). Reduced thymus weight with mild atrophic changes developed in Hartley guinea pigs treated with weekly gavage doses of ≥0.5 μg/kg/day 2,3,7,8-tetraCDF for 6 weeks (Luster et al. 1979a, 1979b). 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), and in C57BL/6fh(J67) mice treated with 300 μg/kg/day 2,3,7,8-tetraCDF by gavage on 22 days in a 30-day period (lower doses not evaluated) (Moore et al. 1979). Thymic atrophic changes, including small lobules without cortices and marked involution, were found in rhesus monkeys that were fed diets containing 2,3,7,8-tetraCDF dosages of 0.21 μg/kg/day for 6 months or 2.1 μg/kg/day for 2 months (McNulty et al. 1981). Effects on the thymus also occurred in offspring of rats exposed during gestation (see Section 2.2.2.6).

Pathological changes in immune system tissues other than thymus were also observed in some of the above studies. These included hypocellularity of bone marrow and lymphoid elements in spleen and Peyer's patches of guinea pigs given single doses of ≥3 μg/kg/day 2,3,4,7,8-pentaCDF or ≥5 μg/kg/day 2,3,7,8-tetraCDF (Moore et al. 1979), and increased extramedullary hematopoiesis in splenic red pulp and occasional atrophic changes in lymph nodes in rats treated with ≥1 μg/kg/day 2,3,4,7,8-pentaCDF for 13 weeks (Pluess et al. 1988a, 1988b; Poiger et al. 1989). There were no treatment-related histological changes in lymph nodes or spleen in rats similarly treated with ≤10 μg/kg/day 1,2,3,7,8-pentaCDF or 1,2,3,6,7,8-hexaCDF or ≤300 mg/kg/day 1,2,3,4,8-pentaCDF. Six weekly doses of ≤1 μg/kg/day 2,3,7,8-tetraCDF caused no changes in spleen weight or histology in guinea pigs (Luster et al. 1979a, 1979b). Spleen weight in mice was unaffected by four weekly ≤100-μg/kg doses of a pentaCDFs/tetraCDFs mixture (Oishi and Hiraga 1980). The ED_{50} for decreased splenic response to intraperitoneally injected sheep red blood cells was 208 μg/kg in mice given a single oral dose of 1,2,3,4,6,7,8-heptaCDF (Kerkvliet et al. 1985).

Limited information on effects of CDFs on immunocompetence is available from above studies. Guinea pigs treated with six weekly doses of 0.5 μg/kg/day 2,3,7,8-tetraCDF had significantly decreased macrophage inhibition index but no significant effect on another cell-mediated immunity indicator, delayed hypersensitivity index (Luster et al. 1979a, 1979b). There were no treatment-related effects on humoral immune function as indicated by serum protein levels (albumin and alpha, beta, and gamma globulins) and IgG antibody responses. Proliferation of lymphocytes following *in vitro* stimulation with the T-lymphocyte mitogen phytohemagglutinin and the B-lymphocyte mitogen lipopolysaccharide were significantly decreased at ≥0.5 μg/kg/day, but the T-lymphocyte mitogen concanavalin A had no effect. 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). In conclusion, the limited number of studies available 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 have been 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. The highest NOAEL values and all reliable LOAEL values for immunological effects in each species and acute- and intermediate-duration categories are recorded in Table 2-l and plotted in Figure 2- 1.

2.2.2.4 Neurological Effects

Various neurological symptoms, including numbness, weakness and neuralgia of limbs, hypesthesia and headaches, are 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 patients examined soon after poisoning (Kuroiwa et al. 1969). Sensory fibers may have been 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 patients, 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 patients (Chen et al. 1985a; Chia and Chu 1984, 1985). Evaluation of 110 patients within 1 year of Yu-Cheng exposure showed abnormally slow sensory nerve (median and ulnar) and motor nerve (tibia1 and peroneal) conduction velocities in ≈44% and 22% of the patients, respectively (Chen et al. 1985a). 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 patients did not show any abnormalities potentially indicative of central nervous system damage (Chia and Chu 1984, 1985). Neurobehavioral deficits have been observed in children born to mothers with Yu-Cheng exposure (see Section 2.2.2.6).

Limited information is available on possible neurological effects of CDFs in animals. Signs of toxicity in Fischer-344 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). Single gavage doses of ≤30 μg/kg 2,3,4,7,8-pentaCDF or ≤15 μg/kg 2,3,7,8-tetraCDF to guinea pigs or ≤6,000 μg/kg 2,3,7,8-tetraCDF to mice produced no histological alterations in the brain during the following 30 days (Moore et al. 1979). 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). The effects observed in the above studies are not necessarily indicative of a direct effect on the central nervous system and could be secondary to other changes (e.g., wasting syndrome, stress) occurring in intoxicated or dying animals. Because more sensitive neurological tests were not performed, insufficient information is available for evaluating the neurotoxic potential of CDF congeners in animals, and effect levels for neurological effects are not recorded in Table 2-l or plotted in Figure 2- 1.

2.2.2.5 Reproductive Effects

Irregular menstrual cycles and abnormal basal body temperature patterns were observed in ≈60% and 85% of female Yusho patients, 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. Fertility, fecundity and rates of spontaneous abortion have not been studied in Yusho and Yu-Cheng patients (Kuratsune 1989; Rogan 1989).

Limited information is available on possible reproductive effects of CDFs in animals. Hypocellularity of the seminiferous tubules was observed in Hartley guinea pigs given single gavage doses of ≥3 μg/kg/day 2,3,4,7,8-pentaCDF or ≥5 μg/kg/day 2,3,7,8-tetraCDF (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). Histology of the testis, ovary, and uterus was normal in Iva:SIV 50 (SD) rats administered dietary dosages of ≤10 μg/kg/day 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF or 1,2,3,6,7,8-hexaCDF, or ≤300 μg/kg/day 1,2,3,4,8-pentaCDF, for 13 weeks (Pluess et al. 1988a, 1988b; Poiger et al. 1989). 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. The animal data suggest that the testis is a target of CDFs, although information on reproductive function is not available and insufficient data preclude assessing species and congener differences. The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and acute- and intermediate-duration categories are recorded in Table 2-l and plotted in Figure 2-l.

2.2.2.6 Developmental Effects

Skin lesions are commonly observed in children born to mothers with Yusho or Yu-Cheng exposure. The dermal changes are consistent with those observed in exposed adults (see Section 2.2.2.2) 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. Eight of 39 hyperpigmented children born to Yu-Cheng-intoxicated mothers died perinatally due to pneumonia, bronchitis, and prematurity (Hsu et al. 1985). Decreased birth weight is another commonly reported effect of Yusho and Yu-Cheng exposure (Funatsu et al. 1971; Lan et al. 1987; Rogan 1989; Taki et al. 1969; Yamaguchi et al. 1971). A health survey of most (117) living children known to have been *in utero* during or after Yu-Cheng exposure found that mean birth weight was decreased ≈15% (Gladen et al. 1990; Rogan et al. 1988). Neurobehavioral assessment based on parental reports showed that 49% of these children were delayed (older) 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). Urinary excretion of total porphyrins was mildly increased in children of Yu-Cheng mothers (Gladen et al. 1988). Immune status was normal in Yu-Cheng children 7-9 years old (Lan et al. 1990).

It is well established that CDFs are teratogenic in rats and mice, inducing dose-related kidney hydronephrosis and/or cleft palate at incidences as high as 100%. Hydronephrosis and cleft palate were induced in C57BL/6N mice by gavage doses as low as 10 and 50 μg/kg/day 2,3,7,8-tetraCDF, respectively (Weber et al. 1984, 1985); 5 and 30 μg/kg/day 2,3,4,7,8-pentaCDF, respectively (Birnbaum et al. 1987a, 1987b); 30 and 100 μg/kg/day 1,2,3,7,8-pentaCDF, respectively (Bimbaum et al. 1987a); and 100 and 300 μg/kg/day 1,2,3,4,7,8-hexaCDF, respectively (Bimbaum et al. 1987a, 1987b). These data are consistent in indicating that hydronephrosis is a more sensitive developmental effect than cleft palate in mice. However, none of the teratology studies in mice examined fetal sites

other than kidney or palate (e.g., nonrenal soft tissues or skeleton), or tested strains other than C57BL/6N. ED_{50} values for hydronephrosis and cleft palate were estimated as 7 and 36 μg/kg/day, respectively, for 2,3,4,7,8-pentaCDF, 54 and 133 μg/kg/day, respectively, for 1,2,3,7,8pentaCDF, and 81 and 342 μg/kg/day, respectively, for 1,2,3,4,7,8hexaCDF (Birnbaum et al. 1987a). Toxic effects generally were not observed in mouse dams or fetuses but occurred in some studies at doses equal to or higher than the lowest doses causing teratogenic effects. Fetal edema is a characteristic fetotoxic effect that has been observed visibly or suggested by increased fetal weight. One exception is a report of increased fetal mortality and hydronephrosis in mice occurring at the same doses (≥250 μg/kg/day) of 2,3,7,8-tetraCDF (Weber et al. 1984), but this was not confirmed in another mouse study by the same investigators using similar doses of this congener (Weber et al. 1985). Administration of 80 μg/kg/day of 2,3,4,7,8-pentaCDF caused hemorrhagic lesions in the placenta of C57BL/6N mice (Khera 1992).

Decreased fetal weight, increased fetal mortality, and cleft palate occurred in fetuses of Fischer-344 rats treated with ≥30, ≥100, and 300 μg/kg/day 2,3,4,7,8-pentaCDF, respectively (Couture et al. 1989). No hydronephrosis or other kidney or nonrenal soft tissue anomalies were observed, although fetal relative thymus and lung weights were decreased at 300 μg/kg/day. Missing or delayed thoracic vertebrae and sternebrae were observed at all dose levels (≥30 μg/kg/day) including controls, and cranial ossification was delayed at 300 μg/kg/day. These changes likely represent delayed development, but assessment is complicated by unreported quantitative data for the effects on the vertebrae and sternebrae. There was some evidence of maternal toxicity (e.g., decreased thymus weight) at all tested doses. The fetal mortality data suggest that rats are more sensitive than mice to fetotoxic effects of CDFs.

Mean relative thymus weight was decreased ≈6%, 14%, and 30% in l-week-old offspring of Wistar SPF rats gavaged with 0.5, 2, or 30 μg/kg/day 2,3,4,7,8-pentaCDF, respectively, on gestation day 16 (Madsen and Larsen 1989). This was accompanied by increased hepatic microsomal enzyme activity, but no other end points were evaluated. Interpretation of this study is complicated by lack of tabulated data and reported statistical analysis in the report, but ATSDR evaluation using the Mann-Whitney test shows that the decreases in thymus weight at ≥2 μg/kg/day are statistically significant. The discrepancy in LOAELs for reduced offspring thymus weight in this and the other rat study (Couture et al. 1989) is likely due to the additional exposure received through nursing, although different days of treatment and interstrain differences in sensitivity could also be factors. As discussed in

Section 2.2.2.3 (Immunological Effects), the thymus is also one of the most sensitive targets of CDFs in adult animals. In conclusion, 2,3,7,8 substituted tetra-, penta-, and hexaCDFs induced hydronephrosis, cleft palate, thymic effects, and/or other developmental changes in animals. Rats appear to be more sensitive than mice, although only two studies were performed with mice and other species were not tested, and 2,3,4,7,8-pentaCDF and 2,3,7,8-tetraCDF were the most potent tested congeners. The highest NOAEL values and all reliable LOAEL values for developmental effects in each species and acute-duration category are recorded in Table 2- 1 and plotted in Figure 2- 1,

2.2.2.7 Genotoxic Effects

The levels of sister chromatid exchanges and chromosome aberrations were examined in peripheral lymphocytes of 12 Yu-Cheng nonsmoker women 5 years after they had consumed rice oil contaminated with CDFs and PCBs (Lundgren et al. 1988). In the presence of α-naphthoflavone, the frequency of sister chromatid exchanges was significantly increased in exposed subjects. This finding was explained by postulating that subjects exposed to PCBs and CDFs have increased concentrations of P-450 monoxygenase in lymphocytes which could result in increased formation or retention of metabolites of α-naphthoflavone causing sister chromatid exchanges. The increase in sister chromatid exchanges was correlated with serum PCB congeners, but not with serum levels of CDFs. Chromosome aberration frequencies were similar in control and exposed populations. No studies were located regarding genotoxic effects in animals after oral exposure to CDFs. Other genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

A retrospective mortality study of 887 male and 874 female patients that were observed for an average of 11 years following official registration as Yusho victims found no significant increase in male deaths (79 observed, 66.13 expected) or female deaths (41 observed, 48.90 expected) from all causes (Kuratsune et al. 1987). Mortality for cancer at all sites, however, was significantly increased in males (33 observed, 15.51 expected, standardized mortality ratio [SMR]=2.13) based on Japanese national rates. This is attributable to significantly increased mortality from liver cancer (9 observed, 1.61 expected, SMR=5.89) and cancer of the lung, trachea, and bronchus (8 observed, 2.45 expected, SMR=3.26). The increased mortality from liver cancer remained statistically significant when based on local death rates (SMR=2.53) and when early liver cancer cases (those occurring <9 years after

poisoning) were excluded (SMR=3.85). However, because the geographic distribution of liver cancer deaths was unexpectedly markedly uneven (there was no significant increase in one of two prefectures), the cancer cannot be conclusively associated with Yusho exposure.

Approximately half of 24 deaths observed in 2,061 Yu-Cheng victims by the end of 1983 were attributed to hepatoma, cirrhosis, or unspecified liver diseases with hepatomegaly (Hsu et al. 1985). Diagnoses were made from clinical symptoms and unspecified laboratory examinations. These findings are inconclusive due to unreported incidences and comparison values and high prevalences of hepatitis B, cirrhosis, and liver cancer in Taiwan (Rogan et al. 1989).

No studies were located regarding cancer in animals after oral exposure to CDFs.

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans after dermal exposure to CDFs.

Mortality occurred in hairless mice that were treated with 1,2,3,4,7,8-hexaCDF in acetone in dermal initiation-promotion studies (Hebert et al. 1990). As detailed in Section 2.2.3.8, a single application of acetone or *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG) initiator to intact uncovered skin was followed by promotion with twice weekly applications of 1,2,3,4,7,8-hexaCDF or 2,3,4,7,8-pentaCDF for 20 weeks. Mice pretreated with acetone or MNNG followed by 3.3 or 33.3 µg/kg/day hexaCDF, respectively, experienced 35% mortality compared to 0% in controls. No significant effects on survival were observed in the mice pretreated with acetone or MNNG and promoted with ≤3.3 µg/kg/day 2,3,4,7,8-pentaCDF. The LOAEL value for death in the acetone pretreated mice is recorded in Table 2-2, but the LOAEL for MNNG pretreatment is not included because of the likelihood of interactions that could increase the toxicity of CDFs.

2.2.3.2 Systemic Effects

NOAEL and LOAEL values for systemic effects of dermal exposure to CDFs are available from an intermediate duration mouse initiation-promotion study that used a single application of MNNG or

TABLE 2-2. Levels of Significant Exposure to CDFs - Dermal

Species	Exposure duration/ frequency	System	NOAEL (µg/kg/day)	LOAEL (effect) Less serious (µg/kg/day)	LOAEL (effect) Serious (µg/kg/day)	Reference	Congener
INTERMEDIATE EXPOSURE							
Death							
Mouse	20 wk 2 d//wk 1x/d				3.3 (35% mortality)	Hebert et al. 1990	hexa
Systemic							
Mouse	20 wk 2 d//wk 1x/d	Gastro		3.3 (mucous cell hyperplasia of glandular stomach)		Hebert et al. 1990	hexa
		Hepatic		3.3 (increased liver weight and hypertrophy)			
		Other		33.3 (8% body weight loss)			
Mouse	20 wk 2 d//wk 1x/d	Gastro	3.3			Hebert et al. 1990	penta
		Hepatic		3.3 (increased liver weight and hypertrophy)			
		Other		3.3 (12% decreased body weight gain)			
Immunological							
Mouse	20 wk 2 d//wk 1x/d				3.3 (thymic and spleen atrophy)	Hebert et al. 1990	hexa
Mouse	20 wk 2 d//wk 1x/d				3.3 (thymic and spleen atrophy)	Hebert et al. 1990	penta
Cancer							
Mouse	20 wk 2d/wk 1x/d				8.3 (CEL; skin proliferative lesions following initiation)	Hebert et al. 1990	hexa

TABLE 2-2. Levels of Significant Exposure to CDFs - Dermal (*continued*)

Species	Exposure duration/ frequency	System	NOAEL (µg/kg/day)	LOAEL (effect) Less serious (µg/kg/day)	LOAEL (effect) Serious (µg/kg/day)	Reference	Congener
Mouse	20 wk 2d/wk 1x/d				33.3 (CEL; skin papillomas following initiation)	Poland et al. 1982	tetra
Mouse	20 wk 2d/wk 1x/d				0.08 (CEL; Skin proliferative lesions following initiation)	Hebert et al. 1990	penta

CDFs = chlorinated dibenzofurans; CEL = cancer effect level; d = day(s); Gastro = gastrointestinal; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; wk = week(s); x = times

hexa = 1,2,3,4,7,8-hexachlorodibenzofuran
penta = 2,3,4,7,8-pentachlorodibenzofuran
tetra = 2,3,7,8-tetrachlorodibenzofuran

acetone as the initiator (Hebert et al. 1990). Effect levels in the acetone pretreated mice are recorded in Table 2-2 but values for MNNG pretreatment are not included for noncancer end points due to concern for possible interactive effects on CDF toxicity.

No studies were located regarding respiratory, cardiovascular, hematological, musculoskeletal, or renal effects in humans or animals after dermal exposure to CDFs.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after dermal exposure to CDFs.

Mucous cell hyperplasia developed in the glandular stomach of hairless mice treated with 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF on intact uncovered skin in dermal initiation-promotion studies (Hebert et al. 1990). As detailed in Section 2.2.3.8, initiation with a single application of acetone or MNNG was followed by promotion with twice weekly applications of 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF for 20 weeks. The incidence of stomach hyperplasia was significantly increased in the mice pretreated with acetone and followed by 3.3 μg/kg/day hexaCDF, but not 3.3 μg/kg/day pentaCDF. Stomach hyperplasia also developed in the mice initiated with MNNG and promoted with 3.3 yg/kg/day pentaCDF or ≥8.3 μg/kg/day hexaCDF. No stomach hyperplasia was observed in control groups. It is not known if CDF ingestion from grooming contributed to exposure.

Hepatic Effects. No studies were located regarding hepatic effects in humans after dermal exposure to CDFs.

Increased relative liver weight and histological hypertrophy were observed in hairless mice treated on intact uncovered skin in dermal initiation-promotion studies (Hebert et al. 1990). As detailed in Section 2.2.3.8, initiation with a single application of acetone or MNNG was followed by promotion with twice weekly applications of 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF for 20 weeks. The hepatic changes were observed in 100% of the mice pretreated with acetone and followed by 3.3 μg/kg/day pentaCDF or 3.3 μg/kg/day hexaCDF, as well as mice initiated with MNNG and promoted with ≥0.08 μg/kg/day pentaCDF or ≥8.3 μg/kg/day hexaCDF. Livers were normal in control groups.

Derma/Ocular Effects. No studies were located regarding dermal or ocular effects in humans after dermal exposure to CDFs.

Dermal initiation-promotion studies were performed using hairless mice that were treated with 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF on intact uncovered skin (Hebert et al. 1990). As detailed in Section 2.2.3.8, initiation with a single application of acetone or MNNG was followed by promotion with twice weekly applications of 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF for 20 weeks. No information was reported on nonproliferative dermal effects in mice pretreated with acetone and followed by either pentaCDF or hexaCDF, so dermal toxicity of CDFs alone (i.e., not initiated by MNNG) cannot be clearly evaluated. Dermal changes of possible relevance to CDFs alone occurred in mice initiated with MNNG and promoted with pentaCDF (0.08-3.3 μg/kg/day) or hexaCDF (8.3-33.3 μg/kg/day). Dermal toxicity was evaluated by a mean score based on number of mice within a group with no, mild, moderate, or severe gross changes. Gross effects ranged from coarse and thickened appearance of skin to desquamation. Histological alterations included epidermal hyperplasia, squamous metaplasia of sebaceous glands, inflammation of dermis and atrophy, or loss of hair follicles and sebaceous glands.

Other Systemic Effects. No studies were located regarding other systemic effects in humans after dermal exposure to CDFs.

Effects on body weight gain occurred in hairless mice that were treated with 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF on intact uncovered skin in dermal initiation-promotion studies (Hebert et al. 1990). As detailed in Section 2.2.3.8, initiation with a single application of acetone or MNNG was followed by promotion with twice weekly applications of 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF for 20 weeks. Weight gain decreased (12.5%) significantly in the mice pretreated with acetone followed by 3.3 μg/kg/day pentaCDF. Those pretreated with acetone followed by 33.3 μg/kg/day hexaCDF lost weight. The mice initiated with MNNG showed decreased weight gain with promotion by 3.3 μg/kg/day pentaCDF or 8.3 or 16.7 μg/kg/day hexaCDF, and weight loss with promotion by 33.3 μg/kg/day hexaCDF.

2.2.3.3 Immunological Effects

No studies were located regarding immunological effects in humans after dermal exposure to CDFs.

Decreased thymus and spleen weights with atrophy occurred in hairless mice that were treated with 2,3,4,78-pentaCDF or 1,2,3,4,7,8-hexaCDF on intact uncovered skin in dermal initiation-promotion studies (Hebert et al. 1990). As detailed in Section 2.2.3.8, initiation with a single application of acetone or MNNG was followed by promotion with twice weekly applications of 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF for 20 weeks. Both relative thymus and spleen weights were significantly reduced in mice pretreated with acetone and followed by 3.3 μg/kg/day pentaCDF or 3.3 μg/kg/day hexaCDF. In mice initiated with MNNG, thymus weight was decreased by promotion with ≥1.7 μg/kg/day pentaCDF or ≥8.3 μg/kg/day hexaCDF, and spleen weight was decreased by promotion with 3.3 μg/kg/day pentaCDF or ≥16.7 μg/kg/day hexaCDF. Prominent lymphoid atrophy in both thymus and spleen at "higher" dosages of pentaCDF and hexaCDF was reported but not detailed. The LOAEL values for immunological effects for each congener in the acetone pretreated mice are recorded in Table 2-2, but values for MNNG pretreatment are not included due to concern for interactive effects on CDF toxicity.

No studies were located regarding the following health effects in humans or animals after dermal exposure to CDFs:

2.2.3.4 Neurological Effects

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

2.2.3.8 Cancer

No studies were located regarding cancer in humans after dermal exposure to CDFs.

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 or 8.3-33.3 μg/kg 1,2,3,4,7,8-hexaCDF for 20 weeks (Hebert et al. 1990). Acetone was used as the vehicle for the MNNG and CDFs. The penta- and hexaCDFs were also tested using acetone as the control initiator at a dose of 3.3 μg/kg/day.

Mice initiated with acetone or MNNG and promoted with acetone were used as controls. There were no significant increases in proliferative lesions of the skin in the mice pretreated with acetone and followed by pentaCDF or hexaCDF, although there was an observation period following treatment. However, proliferative skin lesions developed in 77.8-94.4% and 47.1-89.5% of the mice initiated with MNNG and promoted with ≥0.08 μg/kg pentaCDF or ≥8.3 μg/kg hexaCDF, respectively, compared to 10.5% in the control groups. Most of the lesions were hyperproliferative nodules and squamous cell papillomas. In a similarly designed tumor promotion study using 2,3,7,8-tetraCDF, hairless mice were initiated with a single 5 μmol dermal dose of MNNG in acetone followed by twice weekly dermal applications of ≈33.3 μg tetraCDF/kg in acetone for 20 weeks (Poland et al. 1982). Skin papillomas developed in 100% of the mice, compared to control group incidences of 5% in mice initiated with acetone and promoted with tetraCDF and 0% in mice initiated with MNNG and promoted with acetone. These findings indicate that tetraCDF, pentaCDF, and hexaCDF had skin tumor promotion activity. The cancer effect levels (CELs) for each congener are recorded in Table 2-2.

2.3 TOXICOKINETICS

Data regarding toxicokinetics of CDFs in humans are limited to information derived from cases of accidental ingestion and/or exposure by the inhalation and dermal routes. Humans can absorb CDFs by the inhalation, oral, and dermal routes of exposure. CDFs, when administered orally, are well absorbed by experimental animals, but are absorbed less efficiently when administered by the dermal route. Data regarding absorption in animals after inhalation exposure were not available. Absorption rates depend on the chlorine substitution pattern and vehicle used. Tissue distribution of CDFs is similar in humans and animals. Due to their lipophilic nature, CDFs tend to accumulate in lipid-rich tissues. High amounts of CDFs are usually found in the liver, adipose, skin, and muscle. Accumulation in tissues is strongly dependent on the chlorine substitution pattern, which in turn, determines the rate of metabolism. CDFs are metabolized predominantly by cytochrome P-450 to polar metabolites that undergo glucuronidation. Substitutions in positions 4 and 6 impair biotransformation, so that CDFs with these positions substituted, in addition to lateral substitutions (2,3,7,8), are preferentially retained or excreted unchanged. This is true for humans and animals. Data from animal studies show that fecal excretion of metabolites is the main route of excretion of CDFs, regardless of the route of exposure. The exact mechanism of CDF toxicity is not known. It

has been suggested, however, that the mechanism is related to the enhancement of gene expression triggered by initial binding to a cytosolic Ah receptor.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Quantitative data regarding inhalation absorption of CDFs in humans after controlled inhalation exposure to CDFs were not located. However, absorption of CDFs can be inferred from the fact that CDFs have been detected in tissues and blood of subjects after accidental or occupational exposure to airborne CDFs (Schecter and Ryan 1989; Schecter et al. 1991). These subjects were exposed to soot or dust containing CDFs during clean-up operations following a PCB transformer fire or associated with municipal solid waste incineration. The relative contribution of the dermal route cannot be determined.

No studies were located regarding absorption of CDFs in animals after inhalation exposure to CDFs.

2.3.1.2 Oral Exposure

Indirect evidence of oral absorption of CDFs in humans can be derived from the fact that CDFs were detected in blood and in numerous organs and tissues of subjects who ingested rice oil contaminated with CDFs in the Yusho and Yu-Cheng incidents (Chen et al. 1985b; Masuda et al. 1985).

A recent study examined absorption of CDFs from maternal milk in a 3-month-old infant (Jodicke et al. 1992). Analysis of the milk and the infant's stools showed that some highly chlorinated CDF congeners were more concentrated in the stools than in the milk suggesting that these congeners were less well absorbed or more resistant to enterohepatic circulation than those with lower chlorine content. Results from a balance sheet analysis showed that over 95% of the total CDFs in the milk were removed from the intestinal tract of the infant (Jodicke et al. 1992).

In male Hartley guinea pigs, >90% of a single oral dose of 6 μg of ^{14}C-2,3,7,8-tetraCDF/kg in Emulphor/ethanol/water was absorbed over a 3-day period (Decad et al. 1981a). In female Sprague-Dawley rats administered single doses of three different CDFs mixed in food pellets at 3.5-6.3 μg/kg

body weight, 80% of the 2,3,4,7,8-pentaCDF dose was retained in the liver in 24 hours, compared to 34% for 1,2,3,7,8-pentaCDF and 43% for 1,2,3,6,7,8-hexaCDF (Van den Berg et al. 1989). In this study, liver retention was used as an indirect measure of absorption. When similar doses of the CDFs were administered in peanut oil, the amount of retained 1,2,3,7,8-pentaCDF doubled, the amount of retained 2,3,4,7,8-pentaCDF was unchanged, and the amount of retained 1,2,3,6,7,8-hexaCDF increased to 58%. Excretion data showed that male Fischer-344 rats administered single oral doses of 34, 169, or 338 μg ^{14}C-2,3,4,7,8-pentaCDF/kg in corn oil absorbed >70% of the dose over a 3-day period, regardless of the dose; absorption rate, over the dose range tested, was not dose-related (Brewster and Bimbaum 1987). High absorption (≈90%) was also reported for 2,3,7,8-tetraCDF in male Fischer-344 rats administered a single gavage dose of the CDF in Emulphor/ethanol (Birnbaum et al. 1980).

The limited data regarding oral absorption of CDFs in animals suggest that, in general, these compounds are well absorbed and that absorption efficiency depends on the vehicle and the chlorine substitution pattern. However, clear relationships between structure and absorption cannot be established from the available data, since, for example, peanut oil appeared to facilitate absorption of 1,2,3,7,8-pentaCDF and 1,2,3,6,7,8-hexaCDF, but not of 2,3,4,7,8-pentaCDF (Van den Berg et al. 1989).

2.3.1.3 Dermal Exposure

Quantitative data regarding dermal absorption of CDFs in humans after controlled dermal exposure to CDFs were not located. However, absorption of CDFs can be inferred from the fact that CDFs have been detected in the tissue and blood of subjects after accidental exposure (Schecter and Ryan 1989). These subjects were exposed to soot or dust containing CDFs derived from a PCB transformer fire. Exposure occurred during clean-up operations that followed the fire. In these cases, however, the relative contribution of the inhalation route cannot be determined.

Limited information is available regarding dermal absorption of CDFs in animals. Dermal absorption of 1,2,3,7,8-pentaCDF and 2,3,4,7,8-pentaCDF was studied in male Fischer-344 rats, (Brewster et al. 1989). In these animals, 25% and 34% of a dose of 34 μg/kg of ^{3}H-1,2,3,7,8-pentaCDF and ^{14}C-2,3,4,7,8 pentaCDF in acetone, respectively, was absorbed from the clipped back skin over a 3-day period. In the same time period, 49% of a dose of ^{14}C-2,3,7,8-tetraCDF was absorbed. For these three

CDFs, the percentage of the administered dose absorbed decreased as the applied dose increased. For doses near 300 μg/kg of the three CDFs tested, ≈80% of the radioactivity associated with the application site could be removed by swabbing with an acetone-soaked cotton, indicating that the remaining radioactivity had not penetrated through the dermis. In male Fischer-344 rats, the percentage of the administered dose (34 μg/kg) of ^{14}C-2,3,4,7,8-pentaCDF absorbed through the skin over a 3-day period decreased with age (Banks et al. 1990). The greatest decrease was observed between 10- and 36-week-old rats (22% of the administered dose compared to 15% for the adult rats). When absorption rate was expressed as a function of the applied surface area, in order to eliminate the body weight variable, the mass of 2,3,4,7,8-pentaCDF absorbed by the l0-week-old rats was greater than that observed in 36- and 120-week-old animals.

The available information indicates that over a 3-day period, the rates for dermal absorption of tetraand pentaCDFs in animals are half or less than half those observed for oral absorption.

2.3.2 Distribution

Data from autopsy reports from two adult individuals, not known to have been exposed to high concentrations of CDFs, revealed the presence of CDFs in four tissues: abdominal and subcutaneous fat, liver, muscle, and kidney (Ryan et al. 1985a). The CDFs detected were penta-, hexa-, and heptaCDFs. On a whole weight basis, adipose tissue had the greatest amount of CDFs, followed by liver, muscle, and kidney. The most prevalent CDFs were 1,2,3,4,7,8-hexaCDF and 1,2,3,6,7,8-hexaCDF. When the results were expressed on a lipid basis, the concentration of total CDFs did not vary greatly among tissues. A subsequent report by the same group of investigators showed that CDFs were also present in human adrenal and bone marrow (Schecter et al. 1989a). No CDFs could be detected in brain, pancreas, thymus, heart, and testis of a &month-old infant or a 22-year-old adult (Ryan et al. 1986).

Other distribution studies on CDFs in tissues of infants have been performed. In these cases, exposure to CDFs may have occurred in utero and through breast milk. CDFs were reported in the liver and adipose tissue of a breast-fed infant born to a mother with Yu-Cheng (Masuda et al. 1985). Beck et al. (1990) detected CDFs in the brain, adipose tissue, thymus, spleen, and liver of three infants who died of sudden infant death before reaching 1 year of age. Maternal exposures were not reported. Of the three infants, only one had been breast fed for a significant period of time (≈6 months). The

congeners identified in most tissues of the three infants were 2,3,7,8-tetraCDF, 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, and 1,2,3,4,6,7,8-heptaCDF. The most prevalent were 2,3,4,7,8-pentaCDF and 1,2,3,6,7,8-hexaCDF. On a fat weight basis, the brain and adipose tissue had relatively low levels of CDFs, whereas the liver had the highest levels, in particular in the infant who had nursed. The congeneric composition did not differ among tissues or across infants. Unequivocal placental transfer of CDFs was demonstrated by detecting CDFs in the liver of stillborn infants (Schecter et al. 1990a).

The mechanism by which CDFs cross biological membranes is not known; however, it can be assumed that due to their lipophilic nature, penetration of membranes can be easily accomplished.

2.3.2.1 Inhalation Exposure

Data regarding distribution of CDFs in humans following controlled inhalation exposure were not located. However, as indicated in Section 2.3.2.3, CDFs were detected in samples of adipose tissue in a subject involved in clean-up operations that followed an electrical transformer fire (Schecter and Ryan 1989). The relative contribution of the dermal exposure is not known.
No studies were located regarding distribution of CDFs in animals after inhalation exposure to CDFs.

2.3.2.2 Oral Exposure

Data regarding distribution of CDFs in humans are derived mainly from the Yusho and Yu-Cheng incidents, in which individuals consumed rice oil contaminated predominantly with PCBs and CDFs. The concentration of total CDFs in adipose tissue and liver of deceased Yusho patients ranged from 3 to 25 ppb (Masuda et al. 1985). No CDFs were detected in unexposed individuals in that study; however, subsequent studies using more sensitive analytical methods detected CDFs in tissues of unexposed Japanese and Chinese individuals (Ryan et al. 1987a) (see Section 5.5). In general, the congeners identified in the tissue and blood of Yusho patients consisted of congeners with unsubstituted adjacent carbon atoms, the most prevalent of which was 2,3,4,7,%pentaCDF. The least prevalent was 2,3,7,8-tetraCDF. Similar results were obtained by analyzing adipose and liver tissues of an infant born to a Yu-Cheng mother (indicating *in utero* transfer or through nursing, or both) and in blood of Yu-Cheng patients (Kashimoto et al. 1985; Masuda et al. 1985). Since ≈40 different CDF

congeners were identified in the contaminated rice oil, these results suggest preferential metabolism and retention for certain CDF congeners (see Section 2.3.3). Analyses of tissues of a Yu-Cheng patient who died 2 years after poisoning revealed that the liver had the highest concentration of CDFs (≈35 ppb); the concentration in other tissues was one or more than one order of magnitude lower than in the liver (Chen et al. 1985b). The major CDF congeners retained in the liver were 1,2,4,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, and 1,2,3,4,7,8-hexaCDF. The congeneric profile for tissues other than the liver was essentially similar to that of the liver.

Several studies have examined the distribution of CDFs in animals after oral intake. Three days after administration of single gavage doses of 31 or 306 μg ^{14}C-2,3,7-tetraCDF/kg in Emulphor/ethanol to male Fischer-344 rats, the CDF-derived radioactivity was accumulated in liver (3-5%), fat (4-9%), and skin (1%) (Birnbaum et al. 1980). No significant differences were observed between the two dose levels. Muscle and blood accounted for <1% of the total dose.

Male Fischer-344 rats received a single oral dose of ^{14}C-2,3,4,7,8-pentaCDF at 34-338 μg/kg, and 3 days after dosing, CDF-derived radioactivity was most concentrated in the liver (>50%), followed by adipose (6%), skin (0.9%), and muscle (0.5%) (Brewster and Birnbaum 1987). When expressed as percentage of the dose per gram of tissue, the liver had 5.9% followed by adipose with 0.3%, and adrenal with 0.15%. Regardless of how the results were expressed, tissue distribution was not dose-related, and all other tissues and organs had only traces of radioactivity.

The distribution of CDFs in pregnant C57BW6N mice and in the embryos was examined after oral administration of 800 μg ^{14}C-2,3,7,8-tetraCDF/kg in corn oil to the dams on gestation day 11 (Weber and Birnbaum 1985). Approximately 30% and 0.41% of the dose-derived radioactivity per gram of tissue was found in the dams' livers and blood, respectively (only maternal liver and blood were analyzed), on gestation day 12; these percentages declined by half in both tissues on subsequent days. Elimination half-life from the liver was 1.5 days. Less than 0.01% of the dose was detected in whole embryos at day 12, and no radioactivity could be detected at later times. A dose of 1,000 μg 2,3,7,8-tetraCDF/kg causes 100% cleft palate in mice (Weber and Birnbaum 1985).

Experiments conducted in male Hartley guinea pigs administered 6 μg of labeled 2,3,7,8-tetraCDF/kg by gavage in Emulphor/ethanol/water showed that most of the CDF-derived radioactivity (46%) accumulated in fat 3 days after dosing (Decad et al. 1981a). Liver, muscle, and skin accounted for

≈16% each. After six or seven weekly doses of 2,3,7,8-tetraCDF at 1 µg/kg the distribution of radioactivity in the tissues of guinea pigs was similar to that observed after a single oral dose (Decad et al. 1981a).

Distribution studies suggest that, due to their lipophilic nature, CDFs tend to accumulate in lipid-rich tissues such as skin and adipose. Since tissue levels were determined 3 days after dosing in rats and guinea pigs, early redistribution processes between, for example, liver and other tissues are difficult to ascertain. The two studies in rats, one with 2,3,7,8tetraCDF (Birnbaum et al. 1980) and the other with 2,3,4,7,8-pentaCDF (Brewster and Birnbaum 1987), clearly indicate that a relationship exists between substitution pattern and liver retention; >50% of the 2,3,4,7,8-pentaCDF was retained, compared with only 3-5% for the 2,3,7,8 congener. This is because substitution in position 4 appears to delay metabolic transformation (Burka et al. 1990). This was clearly demonstrated in rats, in which 1,2,3,7&pentaCDF had a liver retention half-life of 3.3 days, whereas that for 2,3,4,7,8-pentaCDF was 108 days (Van den Berg et al. 1989). Similar findings were reported in mice in which the elimination half-times from the liver for 2,3,7,8-tetraCDF and 2,3,4,7,8-pentaCDF were 1.5 days (Weber and Birnbaum 1985) and 65 days (de Jongh et al. 1992), respectively.

2.3.2.3 Dermal Exposure

Data regarding distribution of CDFs in humans following dermal exposure were not located. However, CDFs were detected in samples of adipose tissue in a subject involved in clean-up operations that followed an electrical transformer fire (Schecter and Ryan 1989). The relative contribution of the inhalation route of exposure is not known. Four determinations were made over a period of 3 years starting 2 years after exposure. The most prevalent congeners found (consecutive determinations, ppt on a lipid basis) were 2,3,4,7,8-pentaCDF (84, 52, 46, 54), 1,2,3,4,7,8-hexaCDF (143, 101, 65, 63), 1,2,3,6,7,8-hexaCDF (97, 85, 79, 50), and 1,2,3,4,6,7,8-heptaCDF (55, 29, 39, 32). When expressed on a lipid basis, the concentration of the CDF congeners in serum and adipose tissue was similar.

Tissue distribution of CDFs was studied in male Fischer-rats 3 days after receiving single applications of 31-340 µg/kg of labeled 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, or 2,3,4,7,8-pentaCDF in acetone in a clipped area of the back (Brewster et al. 1989). For example, with the lowest dose, the liver had the most CDF-derived radioactivity per tissue (5.4% for 2,3,7,8-tetraCDF, 4.1% for 1,2,3,7,8-pentaCDF,

14.9% for 2,3,4,7,8pentaCDF), followed by adipose tissue, skin, and muscle. All other tissues (other than liver, adipose, skin, and muscle) had <0.01% of the dose. The relative amounts of radioactivity per tissue decreased as the dose increased, reflecting decreased absorption. Expressed as a percentage of total body burden, 2,3,4,7,8-pentaCDF-derived radioactivity (percentage of absorbed dose) detected in the liver and adipose tissue was 72% and 6.7%, respectively. The concentration of radioactivity per gram of tissue was the greatest in the liver with 2,3,4,7,8-pentaCDF > 2,3,7,8-tetraCDF ≥ 1,2,3,7,8-pentaCDF. Again, as pointed out for oral exposure, these results indicate that liver retention is significant and congener specific, with significantly higher amounts of the pentaCDF substituted in position 4 retained.

No significant age-related changes in the distribution of 2,3,4,7,8-pentaCDF in rats were observed (Banks et al. 1990). For the most part, changes in tissue distribution reflected age-related changes in the total mass of specific tissues and organs.

2.3.2.4 Other Routes of Exposure

The tissue distribution of CDFs after parenteral dosing has been studied in several animal species. The results for these studies show that, in general, the distribution of CDFs in tissues is similar to that observed after oral or dermal administration of CDFs.

In rhesus monkeys administered a single dose of 30.7 μg ^{14}C-2,3,7,8-tetraCDF/kg intravenously the CDF was rapidly cleared from the blood (Birnbaum et al. 1981). A two component exponential decay from blood was observed, with half-lives of 1.5 minutes and 1 hour, respectively. Terminal components of the removal of 2,3,7,8-tetraCDF from the blood were not determined in the study. After 21 days, <10% of the CDF-derived radioactivity remained in the body. When the concentration of CDF was expressed per gram of tissue, the concentrations in liver and fat were 4 times that observed in skin and 12 times that observed in muscle and blood. Of the radioactivity extracted from liver and adipose tissue at day 21 and from blood just after dosing, ~90% appeared to be parent compound. However, 67% of the label remaining in blood at day 21 seemed to correspond to metabolites.

The distribution of radiolabeled 2,3,7,8-tetraCDF has also been studied in rats following intravenous injection, After a single dose of 30.6 μg/kg to male Fischer-344 rats, the blood, liver, fat, skin, and

muscle accounted for >90% of the unexcreted dose of CDF at various times after dosing (Birnbaum et al. 1980). Nearly all the radioactivity detected in tissues was unchanged CDF. Loss of radioactivity from tissues could be described by exponential curves with one or more components. Half-lives for the early components ranged from 0.02 days for blood and muscle to 0.45 days for skin. Late components had half-lives ranging from 0.72 days for muscle to 11.l days for skin. Clearance from fat showed a single component with a half-life of 3.7 days. In other tissues, such as adrenals, kidney, thymus, heart, and lungs, 90% of the radioactivity was cleared within 24 hours; in contrast, the specific activity in the liver decreased only 50% in the same time period.

As with rats and monkeys, intravenous injection of ^{14}C-2,3,7,8-tetraCDF in guinea pigs (6 μg/kg) resulted in preferential accumulation of radioactivity in liver, fat, muscle, and skin (Decad et al. 1981a). Chromatographic analysis of these tissues suggested the presence of only parent compound. Three hours after dosing, a loss of radioactivity from the liver could be accounted for by an increase in adipose and skin. After 1 day, mobilization of fat stores resulted in redistribution of radioactivity into the liver. Accumulation of radioactivity in other tissues was minimal over a 9-day period.

Results in mice were similar to those obtained in other animal species. CDF-derived radioactivity was concentrated in the liver, adipose tissue, skin, and muscle of C57BL/6J and DBA/2J male mice injected with a single intravenous dose of 30.6 μg ^{14}C-2,3,7,8-tetraCDF/kg (Decad et al. 1981b). These tissues accounted for >75% of the injected dose. At all times over a l0-day period (except at day l0), the livers of C57BL/6J mice had more radioactivity than livers of DBA/2J mice (the opposite was observed for fat tissue and muscle), but the half-life elimination of the CDF-derived radioactivity from this organ was I.8 days in both strains. Elimination half-lives from adipose tissue were 6 times longer in DBA/2J mice than in the C57BW6J strain, reflecting the higher fat tissue content in the former strain. Greater than 95% of the radioactivity detected in tissues represented unmetabolized CDF.

2.3.3 Metabolism

No data were located regarding metabolism of CDFs in humans. However, some information can be derived from Yusho and Yu-Cheng patients. These subjects ingested contaminated rice oil in which ≈40 different CDF congeners were identified. As indicated in Section 2.3.2.2, analysis of hepatic adipose tissues of some patients revealed the presence of highly chlorinated congeners and congeners

that lacked adjacent unsubstituted carbon atoms (Chen et al. 1985b; Masuda et al. 1985). This indicates that the presence of unsubstituted adjacent carbon atoms favors metabolism, which is consistent with data for other chlorinated aromatic hydrocarbons. Highly chlorinated congeners have slower metabolic rates, possibly due to steric hindrance.

The metabolic disposition of CDFs in animals has not been extensively studied. However, some generalizations can be made based on the available data. It is generally accepted that biotransformation of CDFs occurs primarily in the liver (Birnbaum 1985; Van den Berg 1989). The major metabolic reactions include hydroxylations with or without dechlorination or migration of substituents from the site of hydroxylation to the adjacent carbon, and oxygen bridge cleavage, followed by glucuronidation. Cytochrome P-450 isoenzymes appear to catalyze the metabolic reactions (Van den Berg 1989).

The major possible metabolic products (specific compounds were not identified) of several CDFs found in rat bile after oral and intravenous dosing of CDFs have been described (Poiger and Pluess 1989). Female Sprague-Dawley derived rats were administered a single oral dose of several tetra- and pentachlorinated CDFs in corn oil. In addition, 1,2,3,6,7,8-hexaCDF and 1,2,3,4,7,8-heptaCDF were injected intravenously. The doses ranged between 0.4 and 3.7 mg/kg. Samples of bile were analyzed for 3-7 days starting 2 hours after dosing. The tetra-substituted CDFs 1,3,7,8-, 2,3,7,8-, and 2,3,6,8- exhibited a fairly high rate of metabolic conversion (no quantitative data reported), and each gave rise to tri- and tetra- hydroxylated and dihydroxylated derivatives. No ring-opened compounds were detected, suggesting that substitution of *ortho* atoms to the oxygen is not important for cleavage of the ether bond in tetraCDFs. A recent study by Burka et al. (1990) identified glucuronide and sulfate conjugates of 4-hydroxy-2,3,7,8-tetraCDF and 3-hydroxy-2,7,8-triCDF as the major biliary metabolites in rats dosed intravenously with 2,3,7,8-tetraCDF.

Among the pentaCDFs, the rate of transformation of 1,2,3,4,8-, 1,2,3,7,8-, and 2,3,4,7,8-pentaCDF was high, moderate, and low, respectively (Poiger and Pluess 1989). The predominant metabolite (out of seven compounds found) of 1,2,3,7,8-pentaCDF was a hydroxy-pentaCDF. According to investigators, formation of 6,7-dihydroxy-pentaCDF may also have occurred. Tetrachlorinated compounds were also identified. The major metabolite (out of 12 compounds found) of 1,2,3,7,8-pentaCDF was a dihydroxy-pentaCDF; other derivatives included monohydroxy-tetra- and pentaCDFs and a trichloro-dihydroxyCDF. Metabolism of 2,3,4,7,8-pentaCDF led to two major compounds (out of 10

compounds found), a methoxy-pentaCDF, and a dimethoxy-pentachlorobiphenyl, the latter formed by ether cleavage. A sulfur containing metabolite was also present. Unmetabolized pentaCDFs were also excreted in the bile. Only a small amount of a hydroxy-pentaCDF was identified from 1,2,3,6,7,8-hexaCDF, whereas no metabolites were detected from 1,2,3,4,6,7-heptaCDF.

No metabolites were detected in urine, feces, liver, and adipose tissue of male Wistar rats given a single gavage dose of 250 mg/kg octaCDF in peanut oil (Veerkamp et al. 1981).

The main conclusions regarding metabolic transformation of CDFs are that chlorine substituents in positions four or six, in addition to the lateral positions, inhibit metabolism more than chlorines in positions one and nine, and that metabolic rate strongly decreases as the number of chlorine atoms increases.

2.3.4 Excretion

Since CDFs have been found in human milk samples from a number of countries (Schecter 1991; Van den Berg et al. 1986), breast feeding is a potential source of excretion (and exposure for the infant) for these compounds. CDFs were reported in the liver and adipose tissue of a breast-fed infant born to a mother with Yu-Cheng (Masuda et al. 1985).

2.3.4.1 Inhalation Exposure

Data regarding excretion of CDFs in humans after the inhalation/dermal route are discussed in Section 2.3.4.3.

No studies were located regarding excretion of CDFs in animals after inhalation exposure to CDFs.

2.3.4.2 Oral Exposure

Limited information is available regarding excretion of CDFs or metabolites in humans after oral exposure to CDFs. Data from Yu-Cheng patients showed that many CDF congeners, which were constituents of the contaminated rice oil, were preferentially excreted, since they could not be detected in tissues of these individuals months or years after exposure (Masuda et al. 1985). For two of the

congeners that were preferentially retained, 2,3,4,7-pentaCDF and 1,2,3,4,7,8-hexaCDF, elimination half-lives of ≈2-2.5 years have been estimated (Ryan et al. 1993).

Analysis of the stools from Yusho patients 22 years after the contamination episode showed a high concentration of penta and hexaCDFs relative to control subjects (Iida et al. 1992). For example, 2,3,4,7,8-pentaCDF was on the average 20 times more concentrated in the stools of Yusho patients than in controls.

Results from a recent study in which CDFs were monitored in an infant's feces after breast feeding suggested that the feces may be the preferred route of elimination of highly chlorinated CDF congeners (Jodicke et al. 1992). No further information regarding excretion could be inferred from this study.

Male Fischer-344 rats excreted ≈70% of ^{14}C-2,3,7,8-tetraCDF-derived radioactivity in the feces over a 3-day period (Birnbaum et al. 1980). The CDF was administered by gavage in Emulphor/ethanol at 31 or 306 ug/kg. In the same time period, urinary excretion accounted for ≈1.5% of the administered dose.

Similar results have been reported in mice (Weber and Birnbaum 1985). Pregnant C57BL/6N mice administered a single dose of 800 μg ^{14}C-2,3,7,8-tetraCDF/kg by gavage in corn oil on day 11 of gestation excreted 80% of the administered dose in the feces over a 3-day period. Urinary excretion accounted for 5.4% of the dose. The estimated whole body half-life was ≈2.6 days.

In contrast to rats and mice, male Hartley guinea pigs excreted 11% of ^{14}C-tetraCDF-derived radioactivity in the feces over the same time period after receiving a gavage dose of 6 μg/kg in Emulphor/ethanol/water (Decad et al. 1981a). Urinary excretion accounted for 3.3% of the administered dose. The fact that 2,3,7,8-tetraCDF is retained for a longer time by guinea pigs, compared to rats and mice, is consistent with the greater toxicity exhibited by this congener in guinea pigs (see Section 2.2).

Studies with other congeners reveal that the extent of excretion is not only species-dependent, but also congener-specific. For example, when single doses between 34 and 338 μg ^{14}C-2,3,4,7,8-pentaCDF/kg were administered to male Fischer-344 rats, ≈30% of the CDF-derived radioactivity was excreted in

the feces over a 3-day period, regardless of the dose (Brewster and Birnbaum 1987). No radioactivity was detected in expired air, and urinary excretion accounted for <0.01% of the dose per day. Analysis of fecal samples 1 day after dosing suggested that >50% of the CDF-derived radioactivity was parent compound; however, 2 days later this fell to 20%. These results, when compared with those obtained with the tetra-substituted congener in rats (Birnbaum et al. 1980), suggest that by adding a chlorine substitute to position four in the CDF ring, excretion rate is decreased by half. As indicated in Section 2.3.3, this is related to the metabolic handling of the two congeners.

In summary, data in rats, guinea pigs, and mice suggest that excretion rates for CDFs after oral dosing are species-dependent and congener-specific. In addition, the relative percentage of derivatives excreted in the feces and urine appears to be species-specific, but the fecal route of elimination is predominant.

2.3.4.3 Dermal Exposure

Data regarding excretion of CDFs in humans exposed by the dermal route were not available. However, relevant information regarding elimination of CDF congeners from adipose tissue can be provided from data on an individual exposed to soot from a transformer accident (Schecter and Ryan 1989). Intake of CDFs resulted most likely from a combination of inhalation and dermal exposure, but the relative contribution of each of these routes is not known. Half-lives for elimination were calculated from four determinations made over a period of 3.5 years, starting 2 years after the accident occurred. Assuming first-order kinetics and subtracting background values, half-lives of 0.3 years, between 1.3 and 1.7 years, and 0.5 years were calculated for 1,2,3,4,6,7,8-heptaCDF, 1,2,3,4,7,8-hexaCDF, and 2,3,4,7,8-pentaCDF, respectively. A more recent publication by the same group of investigators (Schecter et al. 1990b) extended the observations on the same subject over a 6-year period and reported blood-adipose combined half-lives of 4.5, 4.0, 4.9, and 6.8 years for 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, respectively. These values were calculated without accounting for background levels.

Excretion of CDFs was studied in male Fischer-344 rats after receiving single applications of 3-340 μg/kg of labeled 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, or 2,3,4,7,8-pentaCDF in acetone in a clipped area of the back (Brewster et al. 1989). Elimination of CDF-derived radioactivity occurred almost exclusively through the feces. For each congener, the relative amount of radioactivity detected

in the excreta decreased as the dose increased. At the lowest dose tested, fecal excretion accounted for 27% of radioactivity for 2,3,7,8-tetraCDF, 8% for 1,2,3,7,8-pentaCDF, and 0.7% for 2,3,4,7,8-pentaCDF. Within 3 days of dosing, 56%, 32%, and 2% of the respective body burden of 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, and 2,3,4,7,8-pentaCDF had been excreted. Two or more polar metabolites were detected in the feces of rats administered 31 μg 2,3,7,8-tetraCDF/kg and 34 ug 1,2,3,7,8-pentaCDF/kg. Approximately 90% of the 2,3,4,7,8-pentaCDF-derived fecal radioactivity appeared to be parent compound. Excretion parameters for 2,3,4,7,8-pentaCDF-derived radioactivity did not change as a function of age in male Fischer-rats (Banks et al. 1990).

These results are consistent with the view that, due to inhibited metabolism, CDF congeners with substitution in position 4 (2,3,4,7,8-penta) are excreted slower than those with unsubstituted position 4 (2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF). This may be also related to the fact that only parent compound was found in the feces of rats given 2,3,4,7,8-pentaCDF, whereas polar metabolites could be detected in feces of those given 2,3,7,8-tetraCDF and 1,2,3,7,8-pentaCDF.

2.3.4.4 Other Routes of Exposure

In male rhesus monkeys that received a single intravenous injection of 30.7 μg ^{14}C-2,3,7,8-tetraCDF/kg, <10% of the CDF-derived radioactivity remained in the body 21 days after the injection (Birnbaum et al. 1981). The excretion of CDF-derived radioactivity followed a single exponential decay curve both in urine and feces. Half-lives for excretion in urine and feces were 6.2 and 10.3 days, respectively. Over the 21-day period, 8% and 43% of the total dose was excreted in the urine and feces, respectively. Whole body half-life was ≈8 days. Analysis of urine samples revealed at least two polar metabolites and no parent compound. Similarly, feces and bile contained almost exclusively CDF metabolites.

The major route of excretion of ^{14}C-2,3,7,8-CDF in rats treated with a single intravenous dose of the chemical was via the feces (Bimbaum et al. 1980). Five days after dosing, ≈80% of the administered radioactivity had been eliminated through the feces, whereas the majority of the urinary excretion (≈5% of the dose) occurred within the first day. Experiments in rats with cannulated bile ducts showed that enterohepatic circulation does not play a role in the distribution of 2,3,7,8-CDF-derived radioactivity. Half-lives for excretion in urine and feces were 0.3 and 1.8 days, respectively. No parent compound was detected in urine during the 6-day observation period, in bile 3 hours after

dosing, and in feces 2 days after the injection. These results indicate that >99% of the CDF-derived radioactivity excreted from the body consisted of several metabolites and no parent CDF.

In C57BL/6J and DBA/2J male mice, the major route of excretion of ^{14}C-2,3,7,8-tetraCDF-derived radioactivity after a single intravenous dose of 30.6 μg/kg was also the feces (Decad et al. 1981b). Over a l0-day period, >80% of the administered dose in C57BL/6J mice and >55% in DBA/2J mice was excreted in the feces as polar metabolites. Urinary excretion accounted for <20% of the dose in each strain of mice. The nature of the chemicals excreted in the urine shifted from mixed composition (parent compound and metabolites) at early time points to almost all metabolites at day 5. Whole body half-lives were estimated at 2 and 4 days for the C57BW6J and DBA/2J strains, respectively.

In contrast to rats, monkeys, and mice, guinea pigs administered a single intravenous dose of ^{14}C-2,3,7,8-tetraCDF (6 μg/kg) excreted similar amounts of CDF-derived radioactivity in urine and feces, 6.6% of the administered dose in 7 days (Decad et al. 1981a). No CDF-derived radioactivity was detected in bile collected for 4 hours after the injection. In feces, >90% of the radioactivity corresponded to parent compound, whereas one or more polar metabolites were detected in the urine. The estimated whole body half-life for 2,3,7,8-tetraCDF in guinea pigs was ≈20 days.

Several important conclusions can be drawn from studies in which CDFs were administered parenterally. In rats and monkeys given the same congener, fecal excretion is the predominant route of elimination. Whole body half-life is species-dependent, and this appears to be related to toxic potency in different species. This also appears to be valid when comparing toxic potency of different congeners within a species, such that in rats, the more toxic 2,3,4,7,8-pentaCDF has a whole body half-life of 64 days (Brewster and Birnbaum 1987), compared with 8 days for 2,3,7,8-tetraCDF. It should be also pointed out that for congeners with the same number of chlorine substitutions, for example 1,2,3,7,8-pentaCDF (half-life of 7.5 days) and 2,3,4,7,8-pentaCDF (half-life of 64 days), the whole body elimination half-life is determined by the chlorination pattern (due to biotransformation preferences) (Brewster and Birnbaum 1987, 1988).

2.3.5 Mechanism of Action

The mechanism by which CDFs enter the blood stream from the lungs or skin is not known, but it has been suggested that in the gastrointestinal tract, ingested CDFs are incorporated into chylomicra

particles that enter the blood stream (Patterson et al. 1989a). In the blood stream, CDFs are bound to different, very low density lipoproteins, low-density lipoproteins, high-density lipoproteins, and also to protein, most likely prealbumin. In human blood, it has been shown that as the degree of chlorination of the CDF congener increases (from four chlorines up) the percentage of CDF associated with the protein fraction increases, suggesting that higher chlorinated CDFs do not partition according to the lipid content of the fraction (Patterson et al. 1989a). This could indicate the presence of specific interactions between the CDF congeners and the carrier proteins or other proteins.

The mechanism of toxicity for CDFs is not completely understood, but has been extensively studied (Bandiera et al. 1984b; Goldstein and Safe 1989; Mason et al. 1985; Poland and Knutson 1982; Safe 1986, 1990a, 1990b; Skeene et al. 1989). 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 1990b, 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. It is beyond the scope of this profile to discuss these studies in detail. 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.

Many of the CDFs and related compounds bind to a cellular receptor (Ah receptor), which regulates the synthesis of a variety of proteins. 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 a four orders of magnitude range, with 2,3,4,7,8-pentaCDF having the highest affinity (EC_{50}= $1.5x10^{-8}$ M, compared to $1.0x10^{-8}$ 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 suggested the existence of a biological receptor as a mediator in the responses caused by CDFs.

CDFs, as well as the other related halogenated aromatic hydrocarbons, induce a variety of microsomal enzyme activities (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; De Vito 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 1990b; 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 positions 2,3,7, and 8 (dioxin-like) exhibited typical 3-methylcholanthrene (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.

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 end points 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 due to *in vivo* metabolism. A similar CDF congeneric pattern of toxicity was found in splenic response assays in C57BL/6 mice (Davis and Safe 1980; Dickerson et al. 1990) and in thymic atrophy and liver hypertrophy in male Wistar rats (Yoshihara et al. 1981) (see Section 2.4). These results, along with results obtained with other halogenated aromatic hydrocarbons (summarized in Safe 1990b), are consistent with and provide support for the common receptor-mediated mechanism of action.

The expression of the toxic response, which is species and strain dependent, is initiated by the binding of individual congeners with the Ah receptor. The responsiveness of a particular organ or cell depends

on the presence of a functional Ah receptor. Initial binding of a CDF congener to the Ah receptor is followed by an activation or transcription step and subsequent accumulation of occupied nuclear receptor complexes. These complexes interact with a specific deoxyribonucleic acid (DNA) sequence in the CYPlAl gene (which regulates the expression of cytochrome P-450IAl isozymes), changing its secondary and supersecondary structure (Elferinck and Whitlock 1990), which leads to enhancement of the CYPlAl gene expression. A specific nucleotide sequence present in multiple copies to which the nuclear complex binds has been identified (Denison et al. 1989). 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 (see Section 2.4).

2.4 RELEVANCE TO PUBLIC HEALTH

The general population is most likely to be exposed to CDFs by the oral route. Most of the information on human health effects that pertains to CDFs is from studies of people who ingested contaminated rice oil for up to 9-10 months during the Yusho and Yu-Cheng poisoning incidents. These health effects cannot be attributed solely to CDFs due to mixed chemical exposure and possible interactions between CDFs, PCBs, and other components of the contaminated rice oils, but there is sufficient evidence that CDFs are the main causal agents (see Introduction to Section 2.2.2). Although the Yusho and Yu-Cheng studies consist largely of observations on groups that are not very well defined and lack controls and accurate intake data, they do provide a generally consistent picture of the health status of the affected people and an indication of potential effects for the general population who are exposed to low levels of CDFs. Manifestations of the Yusho and Yu-Cheng outbreaks include serious health effects such as severe skin lesions (e.g., persistent acneform eruptions, hyperpigmentation) and ocular signs (e.g., hypersecretion of eyelid glands), increased susceptibility to respiratory infection (e.g., chronic bronchitis), and neurological symptoms and signs (e.g., limb numbness, reduced nerve conduction velocities, delayed neurobehavioral development). Less serious effects observed in Yusho and Yu-Cheng patients include mild hematological changes (e.g., anemia) and clinically insignificant hepatic alterations (e.g., changes in ultrastructure and serum triglycerides). Some of these effects, particularly dermal, ocular, and neurobehavioral manifestations, also occurred in children borne by exposed mothers. Some effects of CDFs in treated animals are consistent with and supportive of the human data, although types and sensitivities of specific end points examined often differed in humans and animals and among animal species, and animals studies may have used near

lethal or lethal doses. Many of the toxicity studies of CDFs in animals have involved acute- or intermediate-duration oral exposure, although one intermediate-duration dermal study is available, and most tested the 2,3,7,8-tetraCDF and 2,3,4,7,8-pentaCDF congeners. Effects of oral CDFs in animals that have not been observed or clearly discerned in exposed humans include moderately severe hepatotoxicity, renal effects, severe body weight loss (wasting syndrome), thymic atrophy, possible testicular toxicity, and developmental effects including hydronephrosis and cleft palate. Some of these changes occurred only at near-lethal levels of exposure, but immunologic effects are particularly sensitive, based on thymic changes in both adult and developing animals at low doses. Few studies have investigated the carcinogenicity of CDFs but there is evidence from the dermal study and parenteral injection studies that CDFs can promote development of tumors initiated by other chemicals.

Limited information on human health effects of probable combined dermal and inhalation exposure to CDFs and other chemicals is available from studies related to an electrical transformer fire in Binghamton (New York) in 1981 (Fitzgerald et al. 1986, 1989; Schecter 1983, 1986, 1987; Schecter and Charles 1991; Schecter and Tiernan 1985; Schecter et al. 1985a, 1985b). Dielectric fluid composed of 65% PCBs (Aroclor 1254) and 35% polychlorinated benzenes was pyrolyzed leading to the formation of a fine, oily soot, which was distributed throughout the building via ventilation shafts. The soot contained high levels PCBs, CDFs, chlorinated dibenzo-*p*-dioxins (CDDs), chlorinated biphenylenes, and other chemicals, including average concentrations of 199 ppm 2,3,7,8-tetraCDF and 3 ppm 2,3,7,8-TCDD, but there was wide variation in the quantification of the contaminants. Firefighters, police officers, cleanup workers, and other personnel were exposed for a few minutes to >l,000 hours (median 8 hours). Medical surveillance was performed on 482 potentially exposed people 9-12 months after the fire, including symptomology and physical examinations of 147 people who were in the building for ≥25 hours (Fitzgerald et al. 1986). Exposure was positively related to mean serum PCB levels, but means and individual values were within the range reported by other studies of people with no unusual exposures. Follow-up health evaluations were performed ≈3 years after exposure (Fitzgerald et al. 1989). More than 96% of the original participants were followed, and loss to follow-up was greatest among people who had either the least potential for exposure or lower mean PCB concentrations. Health effects reported in people exposed during the Binghamton State Office Building incident include frequent coughing, nonspecific gastrointestinal symptoms, muscle pain, mild hepatic effects (e.g., elevated levels of serum liver enzymes, ultrastructural alterations), skin abnormalities (e.g., rashes, acne-like lesions [not chloracne], skin color changes), unintentional weight loss, and neurological symptoms (e.g., numbness in extremities, dizziness) (Fitzgerald et al. 1986,

1989; Schecter et al. 1985a, 1985b). Interpretation of these findings is complicated by low incidences, small sample size, short latency time, unknown exposure levels, intakes that probably were low in most cases, possibility of recall bias, subjective nature of some of the effects, intervening effects of stress, combined routes of exposure, and use of various degrees of protection (air packs, protective clothing) by people with the greatest potential for exposure. Another important study limitation is that all persons who felt they may have had exposure were included, despite no evidence of exposure in many of these persons. Due to the co-exposure to CDFs and other toxic chemicals and lack of confirmed doses of these chemicals, health effects cannot be attributed specifically to CDFs or any of the other components of the soot.

Animal studies show that many of the toxic effects attributable to CDFs, including chloracne, immunotoxicity, inhibition of body weight gain, hepatic changes, and teratogenicity, appear to be mediated by a common mechanism of toxicity that involves a specific cytosolic molecular receptor (Ah receptor) (see Section 2.3.5). Because this mechanism also mediates many of the toxic effects of chlorinated dibenzo-*p*-dioxin (CDD) and PCB congeners, which are structurally similar to CDFs, data on toxicity and related issues (e.g., species differences in sensitivity) for CDDs and PCBs are relevant to CDFs. CDDs and PCBs are evaluated in other ATSDR toxicological profiles (ATSDR 1993, 1994), but selected general data for these chemicals are presented in this section to corroborate effects of CDFs. Numerous factors influence the toxicity of CDFs; some of these factors include differences in absorption, distribution, and retention among animal species (see Section 2.3). However, at the tissue level, the toxic potency for each individual congener is determined by the magnitude of the response that is initiated by the binding of a specific congener with the Ah receptor. As discussed in Section 2.3.5, the binding affinity, in turn, is determined by the substitution pattern of the congener. For many 2,3,7,8-substituted congeners and congeners with less than four lateral substituents, there is a qualitative correlation between their structure-binding and structure-toxicity relationships (Mason et al. 1985). The tissue-specific toxicological effects exhibited by individual CDF congeners (i.e., for 2,3,4,7,8-pentaCDF, a 30-fold difference between ED_{50} values for immunotoxicity and teratogenicity in mice) may not reflect differences in receptor affinity, but rather differences in the battery of enzymes expressed or repressed as a result of the binding with the receptor. Although there is no direct evidence with CDFs to support this view, data with 2,3,7,8-TCDD strongly suggest that this may be the case (Gasiewicz and Rucci 1984). Only a few oral studies have been performed with CDFs other than 2,3,7,8-pentaCDF and 2,3,4,7,8-pentaCDF, but results of these studies indicate that these are among the most toxic congeners. *In vitro* and acute parenteral structure-activity studies, which

typically evaluated sensitive end points such as Ah receptor binding, induction of hepatic microsomal enzymes (e.g., AHH and EROD), body weight loss, and thymic atrophy, and tested a broad spectrum of congeners, have shown that tetra- to hexaCDFs that are fully substituted in the lateral two, three, seven, and eight positions are the most active congeners (Bandiera et al. 1984b; Holcomb et al. 1988; Mason et al. 1985; Safe 1990a; Safe et al. 1986). These studies also indicate that effects of CDFs are generally independent of exposure route. Evaluations of *in vivo* and *in vitro* data for derivation of toxicity equivalent factors (see following paragraph) have shown that 2,3,4,7,8-pentaCDF is more toxic than 2,3,7,8-tetraCDF and other CDF congeners. Additionally, 2,3,7,8-tetraCDF is not expected to be an important contributor to human CDF toxicity because 2,3,7,8-tetraCDF is more rapidly metabolized than the other 2,3,7,8-substituted CDFs (see Section 2.3.3).

People are environmentally exposed to mixtures of halogenated aromatic hydrocarbons, of which various CDFs are constituents, rather than to single CDF congeners. In particular, CDDs frequently occur with CDFs in the environment, and due to the common mechanism of toxicity, total toxicity is from both together. The total toxicity is not necessarily the sum of the total individual congener toxicities since CDFs and CDDs compete for the same receptor and thus, nonadditive behavior may occur. The public and toxicological concerns resulting from exposure to CDFs and structurally related CDDs, as well as the gaps in available information with which to evaluate the human health potential from exposure to CDFs and CDDs, are well recognized (Ahlborg et al. 1992; EPA 1989; McFarland and Clarke 1989; Safe 1990a, 1991). In response to this problem, the EPA Chlorinated Dibenzo-*p*-dioxins/Chlorinated Dibenzofurans Technical Panel of the Risk Assessment Forum recommends an interim method for assisting in estimating the risk from exposure to these chemical mixtures that can be used until the data gaps are filled (Barnes 1991; Bellin and Barnes 1991; EPA 1989). This procedure generates toxicity equivalence factors (TEFs) based on congener-specific data and the assumption that Ah receptor-mediated toxicity is additive. The TEF scheme compares the relative toxicity of individual CDFs and CDDs congeners to that of 2,3,7,8-TCCD, which is the most toxic and extensively studied of these halogenated aromatic hydrocarbons. The TEFs presented in Table 2-3 provide a means of relating toxicity data for CDFs and CDDs, which frequently occur together, to an equivalent level of 2,3,7,8-TCDD. The TEF for 2,3,7,8-TCDD is defined as unity, whereas TEF values for all other CDF and CDD congeners are less than one (zero has been assigned to all non-2,3,7,8-substituted congeners), thus reflecting the lower toxic potency of most CDF and CDD congeners. 2,3,4,7,8-PentaCDF is the most toxic CDF congener with a TEF five times higher than 2,3,7,8-tetraCDF. The TEFs thus generated can be used, assuming additivity of the toxic response, for

TABLE 2-3. Recommended Toxicity Equivalency Factors (TEFs) for CDFs and CDDs[a]

Compound (CDFs)		Compound (CDDs)	
CDFs	EPA current recommended values	CDDs	EPA current recommended values
monoCDFs	0	monoCDDs	0
diCDFs	0	diCDDs	0
triCDFs	0	triCDDs	0
2,3,7,8-tetraCDF	0.1	2,3,7,8-TCDD	1
other tetraCDFs	0	other tetraCDDs	0
1,2,3,7,8-pentaCDF	0.05	2,3,7,8-pentaCDD[b]	0.5
2,3,4,7,8-pentaCDF	0.5	other pentaCDDs	0
other pentaCDFs	0		
2,3,7,8-hexaCDF[b]	0.1	2,3,7,8-hexaCDDs[b]	0.1
other hexaCDFs	0	other hexaCDDs	0
2,3,7,8-heptaCDFs[b]	0.01	2,3,7,8-heptaCDDs[b]	0.01
other heptaCDFs	0	other heptaCDDs	0
octaCDF	0.001	octaCDD	0.001

[a]Derived from EPA 1989
[b]Any isomer that contains chlorine in the 2,3,7,8-positions

CDDs = chlorinated dibenzo-*p*-dioxins; CDFs = chlorinated dibenzofurans; NR = not reported; TEFs = toxicity equivalence factors; TCDD = tetrachlorodibenzo-*p*-dioxin

estimating the toxicity of an environmental mixture containing a known distribution of CDFs and/or CDDs relative to that of 2,3,7,8-TCDD (for further information see Section 2.6). The TEF values will change over time as new toxicity data are obtained. The TEF approach facilitates site-specific assessments that account for changes in congener composition due to differential environmental partitioning and transformation, as well as differences in congener profiles between sites and co-exposure to CDDs. The approach is controversial, however, because it is only useful for those congeners which exhibit dioxin-like activity and is not adequately validated (Brown et al. 1992; De Vito et al. 1993; Eadon et al. 1986; Harper et al. 1993; Neubert et al. 1992; Pluess et al. 1988b; Poiger et al. 1989; Safe 1992).

Minimal Risk Levels for CDFs

Inhalation MRLs

No MRLs have been derived for inhalation exposure to CDFs because human and animal data for all durations are lacking.

Oral MRLs

- An MRL of 0.001 μg/kg/day has been derived for acute-duration oral exposure (14 days or less) to 2,3,4,7,8-pentaCDF.

The acute oral MRL was based on a LOAEL for mild thymic lymphoid hypoplasia identified in groups of 6 male Hartley guinea pigs (age 3-4 weeks) that were observed for 30 days following treatment with a single gavage dose of 0, 1, 3, 10, or 30 μg/kg 2,3,4,7,8-pentaCDF in corn oil (Moore et al. 1979). The 3 μg/kg/day dose is the LOAEL as histological examinations were not performed at the lowest dose. The LOAEL is supported by evidence from other studies in guinea pigs, rats, mice, and monkeys that the thymus is a sensitive indicator of immunologic effects of CDFs following acute or intermediate duration oral exposure (Brewster et al. 1988; Kerkvliet et al. 1985; Luster et al. 1979a, 1979b; Moore et al. 1979; McNulty et al. 1981; Oishi and Hiraga 1980; Oishi et al. 1978; Pluess et al. 1988b; Poiger et al. 1989). Thymic atrophic changes manifested as histologic alterations and/or decreased organ weight were characteristic effects in these studies.

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- An MRL of 0.00003 µg/kg/day has been derived for intermediate-duration oral exposure (15-364 days) to 2,3,4,7,8pentaCDF.

The intermediate oral MRL was based on a LOAEL for hepatic effects (increased serum bilirubin, decreased serum triglycerides) identified in groups of six male and six female 1va:SIVSO (SD) rats (age ≈7 weeks) that were fed diets providing estimated dosages of 0, 0.1, 1, or 10 pg/kg/day for 13 weeks (Pluess et al. 1988a; Poiger et al. 1989). The LOAEL of 0.1 µg/kg/day is supported by evidence from other animal studies that the liver is a target of CDFs following acute- and intermediate-duration oral exposure (Ahlborg et al. 1989; Brewster et al. 1988; Doyle and Fries 1986; McNulty et al. 1981; Moore et al. 1979; Oishi and Hiraga 1978; Oishi et al. 1978; Pluess et al. 1988b; Poiger et al. 1989). Typical hepatic changes observed primarily in rats and monkeys include 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.

The acute- and intermediate-duration MRLs discussed above are for 2,3,4,7,8-pentaCDF, which is more toxic than some other CDF congeners. Therefore, applying these MRLs to other CDFs may lead to overestimating actual risks.

An MRL for chronic-duration oral exposure was not derived for CDFs because human and animal data are lacking for this exposure category.

Death. Limited information is available on mortality in humans exposed to CDFs. An epidemiological study of the Yusho incident showed no increased noncancer mortality and an inconclusive increase in liver cancer mortality (Kuratsune et al. 1987). Deaths occurred in ≈1% of Yu-Cheng victims, apparently due to nonmalignant or malignant liver disease in half the cases (Hsu et al. 1985), but comparison rates were not reported. Data from these studies are insufficient for determining if low level exposure to CDFs by oral or other routes is likely to cause death in humans.

Oral studies in animals indicate that CDFs are extremely toxic, causing death in the ug/kg range after acute and intermediate duration exposure (Brewster et al. 1988; Ioannou et al. 1983; Moore et al. 1976, 1979; Pluess et al. 1988a, 1988b; Poiger et al. 1989). The guinea pig and monkey are especially sensitive species and congeners substituted in the 2,3,7,8-positions, particularly 2,3,4,7,8-pentaCDF and

2,3,7,8-tetraCDF, are most toxic. Animals usually do not die for many days following exposure to a single oral dose of CDFs (30-day observation periods are common in lethality assays), indicating that delayed toxicity may be a concern in acutely exposed humans. Acute and subchronic gavage studies with guinea pigs suggest that total dose administered may be a more important determinant of lethality than size of a single dose or frequency of exposure (Luster et al. 1979a, 1979b; Moore et al. 1979). Death was usually preceded by a weight loss (wasting syndrome), but insufficient information is available to determine the cause of death. An intermediate duration dermal exposure study showed that 1,2,3,4,7,8-hexaCDF was more toxic than 2,3,4,7,8-pentaCDF in mice when applied in acetone vehicle (Hebert et al. 1990). Insufficient information is available for these isomers to compare dermal and oral toxicity, but dermal absorption is likely to be less than half of oral absorption (see Section 2.3.1) and dermal absorption from acetone vehicle is likely to be much higher than from soil. The deaths observed in animals orally or dermally exposed to CDFs suggest that death could also occur in humans if exposure is high enough.

Systemic Effects

Respiratory Effects. Chronic bronchitis and related effects (e.g., cough, expectoration) were observed in many Yusho and Yu-Cheng patients (Kuratsune 1989; Nakanishi et al. 1985; Rogan 1989; Shigematsu et al. 1971, 1977). The bronchitis, which was generally attributed to severe respiratory infection resulting from lowered resistance to illness (i.e., secondary to immunological effects), differed from usual bronchitis (e.g., no crackles) and only gradually improved following exposure. No pulmonary histological changes developed, however, in animals that were treated with single nonlethal (guinea pigs, mice) or lethal (guinea pigs, rats) doses of 2,3,4,7,8-pentaCDF or 2,3,7,8-tetraCDF .(Brewster et al. 1988; Moore et al. 1979). Information on respiratory effects of CDFs in animals after intermediate or chronic duration exposure is not available. Interpretation of the human data is complicated by co-exposure to PCBs, although there is evidence that PCBs and CDDs can induce respiratory effects (ATSDR 1993, 1994), and the lack of longer duration animal studies. The existing data indicate a potential for respiratory effects in humans exposed to CDFs when exposure levels are high enough.

Cardiovascular Effects. No information is available on cardiovascular effects of CDFs in humans. Hemorrhages occurred under the nails of rats, in the stomach of monkeys, and in the adrenals of guinea pigs given single lethal oral doses of 2,3,7,8-tetraCDF and/or 2,3,4,7,8-pentaCDF (Brewster et

al. 1988; Moore et al. 1979). This is suggestive of a general hemorrhagic effect in dying animals. Histology of the heart was normal in animals that were treated with single nonlethal (guinea pigs, mice) or lethal (guinea pigs, rats) gavage doses of 2,3,4,7&pentaCDF or 2,3,7,8-tetraCDF (Brewster et al. 1988; Moore et al. 1979). Similarly, cardiac histology was not altered in rats fed nonlethal doses of 1,2,3,7,8-pentaCDF, 1,2,3,4,8-pentaCDF or 1,2,3,6,7,8-hexaCDF, or a lethal dose of 2,3,4,7,8-pentaCDF, for 13 weeks (Pluess et al. 1988a, 1988b; Poiger et al. 1989). Chronic studies have not been performed and there is no information on effects of CDFs on cardiovascular function, although 2,3,7,8-substituted CDDs can induce cardiovascular effects (ATSDR 1994). Based on the available information, it is not known whether populations exposed to CDFs near hazardous waste sites may develop cardiovascular effects. It has been suggested that hemorrhages observed in animals exposed to CDFs and related halogenated aromatic compounds are due to impaired clotting, resulting from decreased numbers of platelets, but there are few data in support of this mechanism (McConnell 1989).

Gastrointestinal Effects. No information was located regarding gastrointestinal effects of CDFs in humans other than symptoms such as vomiting and diarrhea following exposure during the Yusho incident (Kuratsune 1989). Gastrointestinal tract histology was normal in some animal species that were administered single nonlethal (guinea pigs, mice) or lethal (guinea pigs) gavage doses of 2,3,4,7,8-pentaCDF or 2,3,7,8-tetraCDF (Moore et al. 1979). However, near-lethal or lethal single gavage doses of these congeners produced gastric effects in rats (epithelial hyperplasia) and monkeys (e.g, focal ulceration and mucosal cysts) (Brewster et al. 1988; Moore et al. 1979). Similar gastric changes also occurred at lethal doses in intermediate duration diet studies with 2,3,7,8-tetraCDF in monkeys (mucosal cysts) (McNulty et al. 1981) and 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF in dermal studies with mice (mucous cell hyperplasia) (Hebert et al. 1990). Gastrointestinal effects, including mucosal changes that progress to stomach ulcerations and hemorrhages, are also common in monkeys after exposure to 2,3,7,8-TCDD or PCBs (ATSDR 1993, 1994), suggesting that primates may be particularly susceptible to CDF-induced gastrointestinal toxicity. No specific information on mechanism(s) was located. The animal data suggest, however, that the symptoms reported by Yusho patients could possibly reflect a direct gastrointestinal effect of 2,3,7,8-substituted CDFs rather than central nervous system or general toxicity of CDFs, and indicate that there is a possibility of gastrointestinal effects occurring in populations exposed to CDFs near hazardous waste sites.

2. HEALTH EFFECTS

Hematological Effects. Mild normocytic anemia and leukocytosis are fairly consistent findings in Yu-Cheng patients (Rogan 1989). Alterations indicative of mild anemia (decreased hemoglobin concentration with generally unchanged red blood cell count) are the only hematological effects consistently observed in animals exposed to CDFs. Alterations have been observed in single dose studies with 2,3,4,7,8-pentaCDF or 2,3,7,8-tetraCDF in rats, mice, and monkeys (not in guinea pigs), and in intermediate-duration studies with 2,3,4,7,8-pentaCDF, 1,2,3,7,8-pentaCDF or 1,2,3,6,7,8-hexaCDF in rats, but not with 2,3,7,8-tetraCDF in mice, guinea pigs, or monkeys (Brewster et al. 1988; Luster et al. 1979a, 1979b; McNulty et al. 1981; Moore et al. 1979; Pluess et al. 1988a, 1988b; Poiger et al. 1989). Intermediate-duration exposure to a mixture of various tetra-, penta-, and hexaCDF caused hemolytic anemia in rats (Oishi and Hiraga 1978; Oishi et al. 1978). Insufficient information is available to explain the variations in response, but total doses of each congener could be a factor, as studies with related halogenated aromatic compounds indicate that anemia is related to dose-level and duration of exposure (McConnell 1989). Studies with 2,3,7,8-TCDD indicate that monkeys may be highly sensitive to hematological effects of CDFs (ATSDR 1994). No treatment-related hematologic alterations occurred in the only animal study of a non-2,3,7,8-substituted CDF congener (1,2,3,4,8-pentaCDF in rats) (Pluess et al. 1988b; Poiger et al. 1989). Anemia observed in animals exposed to CDFs and related halogenated aromatics has been postulated to be due to toxic effects on erythropoiesis (McConnell 1989). The available evidence indicates that it is possible that hematological effects can occur in populations exposed to 2,3,7,8-substituted CDFs near hazardous waste sites.

Musculoskeletal Effects. Musculoskeletal effects have not been reported in Yusho or Yu-Cheng victims (Kuratsune 1989; Rogan 1989). Reduced muscle mass with no altered muscle histology was observed in guinea pigs following single gavage doses of 2,3,7,8-tetraCDF or 2,3,4,7,8-pentaCDF (Moore et al. 1979), but this is likely a manifestation of the general wasting syndrome. The animal data, therefore, provide an insufficient basis for assessing if the musculoskeletal system is a potential target of CDFs.

Hepatic Effects. The liver is a target organ of CDFs in humans and animals. Various hepatic effects have been observed in humans exposed during Yusho, but increased SGOT and SGPT levels, increased serum triglycerides with unchanged serum cholesterol and increased urinary excretion of uroporphyrin appear to be the most consistent changes (Chang et al. 1980; Gladen et al. 1988; Kuratsune 1989; Lu et al. 1980; Okumura et al. 1979; Rogan 1989; Uzawa et al. 1969). A marked

elevation in serum triglycerides is one of the few abnormal findings peculiar to Yusho and Yu-Cheng, but the mechanism of the increase is unknown (Kuratsune 1989; Rogan 1989). Ultrastructural changes, particularly endoplasmic reticulum alterations probably indicative of microsomal MFO enzyme induction and mitochondrial alterations, appear to be the predominant hepatic morphological finding in Yusho patients. There is no evidence that the above hepatic effects observed in humans are clinically significant.

CDFs have induced generally 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; Poiger et al. 1989). Tetra-, penta-, and hexaCDF congeners substituted in the 2,3,7,8 positions were more hepatotoxic than congeners not substituted in these positions. This pattern of toxicity has also been demonstrated in acute intraperitoneal and *in vitro* structure-activity relationship studies that evaluated induction of hepatic microsomal MFO enzymes (e.g., AHH, EROD) in rats (Bandiera et al. 1984b; Holcomb et al. 1988; Mason et al. 1985; Safe et al. 1986). As discussed in Section 2.3.5, 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 P-450IA-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 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). The lowest intermediate duration dose observed to cause hepatic effects was 0.1 μg/kg/day 2,3,4,7,8-pentaCDF, which increased serum bilirubin and decreased serum triglycerides in rats (Pluess et al. 1988a; Poiger et al. 1989). This LOAEL is used as the basis for an intermediate-duration MRL for oral exposure and also caused decreased thymus weight in rats (see Immunological

Effects). Intermediate-duration dermal exposure to 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF induced increased relative liver weight and liver hypertrophy in mice (Hebert et al. 1990). Since there is suggestive human and conclusive animal evidence that the liver is a target organ of CDFs by different routes of exposure, it is possible that hepatic effects can occur at sufficiently high exposure levels in populations exposed to CDFs near hazardous waste sites.

Renal Effects. No effects on the kidney have been reported in humans exposed during the Yusho or Yu-Cheng incidents (Kuratsune 1989; Rogan 1989). Renal toxicity of CDFs in animals was investigated in several oral studies which found changes only at acute lethal levels of 2,3,7,8-tetraCDF and 2,3,4,7,8-pentaCDF (Brewster et al. 1988; Moore et al. 1979). Effects included increased blood urea nitrogen and/or hyperplasia of the renal pelvis, ureter, and bladder in guinea pigs, rats, or monkeys. Hyperplasia was not observed in rats, which is consistent with studies of related chlorinated halogenated compounds (McConnell 1989). Based on these studies, it has been postulated that the hyperplastic responses may be related to species differences in the rate and route of elimination of these chemicals from the body (i.e., proportion of a given dose excreted via urine). Developing kidneys are also a target of CDFs as shown by an alteration in the ureteral epithelium (hydronephrosis) in mice induced by *in utero* exposure to CDFs (see Developmental Effects). The animal data suggest that there is a possibility that mild renal and urinary tract effects could develop in humans if exposure is high enough.

Dermal/Ocular Effects. Effects in the skin and eyes were the most obvious and frequent manifestations of toxicity following oral exposure during the Yusho and Yu-Cheng incidents (Fu 1984; Kuratsune 1989; Lu and Wu 1985; Rogan 1989). Characteristic skin changes included follicular plugging in pilosebaceous orifices, acneform eruptions, dark colored pigmentation frequently in the gingival and buccal mucosa, lips and nails, and deformed nails. Skin abnormalities include acne-like lesions (not chloracne). Examinations 16 years after the Yusho outbreak showed that more than half of the patients still exhibited some dermal signs (Toshitani et al. 1985). Eye discharge and other severe ocular effects occurred during the acute phase of the Yusho and Yu-Cheng syndrome, such as meibomian gland (eyelid) changes including enlargement, irritation and hypersecretion, and abnormal pigmentation of the conjunctivae and eyelids. Improvement of the dermal and ocular effects was gradual, apparently because of slow release of CDFs from body adipose stores. Of 75 Yusho patients examined ≈l0 years after the outbreak, 84% and 43% still showed abnormal changes in the Meibomian glands (e.g., atrophy, secretion) and pigmentation of the conjunctivae and eyelids,

respectively (Kono and Yamana 1979). Considering an estimated elimination half-life of 1.5 years for two toxic congeners that were preferentially retained in Yu-Cheng patients (Ryan et al. 1990), it is evident that persistent dermal and ocular effects are associated with small body burdens of CDFs.

Average body burdens of CDFs associated with chloracne in Yusho and Yu-Cheng victims have been estimated (Ryan et al. 1990). Using Yusho oil consumption data and assuming a clearance half-life of 1.5 years for the two most toxic congeners (2,3,4,7,8-pentaCDF and 1,2,3,4,7,8-hexaCDF), the body burden associated with chloracne in Yusho victims was estimated as 5.9 μg/kg expressed as a 2,3,4,7,8-pentaCDF equivalent (PEQ) amount. This is somewhat higher than a body burden of 4.4 μg PEQ/kg estimated to produce the first clinical signs of Yusho illness (nausea and anorexia). Using measured blood levels of CDFs in Yu-Cheng victims with chloracne and assuming 2,3,4,7,8-pentaCDF:1,2,3,4,7,8-hexaCDF ratios of 1:l in blood and 5:l for relative toxicity, the body burden associated with chloracne in Yu-Cheng victims was estimated as 4.0 μg PEQ/kg. Using a TEF-based analysis, this Yu-Cheng body burden was shown to be comparable to adipose tissue levels of 2,3,7,8-TCDD known to cause chloracne in monkeys. The TEF principle is discussed in the introduction to Section 2.4.

Single-dose and intermediate-duration oral studies have shown dermal and ocular effects in monkeys exposed to 2,3,7,8-tetraCDF, but not in guinea pigs exposed to 2,3,7,8-tetraCDF or 2,3,4,7,8-pentaCDF (McNulty et al. 1981; Moore et al. 1979). Effects were progressive, dose-related, and consistent with those observed in Yusho and Yu-Cheng victims, including facial and periorbital edema, exudate and occlusion of meibomian and ceruminous (ear canal) glands, hyperkeratotic sebaceous gland ducts, follicular orifices and nail beds, and loss of facial and body hair and nails. Monkeys also have been shown to be very sensitive to similar dermal effects induced by oral exposure to 2,3,7,8-TCDD or PCBs (ATSDR 1993, 1994). Skin changes also developed in mice that were dermally treated with a single dose of tumor initiator (MNNG) followed by intermediate duration application of 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF (Hebert et al. 1990). These included epidermal hyperplasia, squamous metaplasia of sebaceous glands, inflammation of dermis, and atrophy or loss of hair follicles and sebaceous glands. Although application of CDFs without initiator was not performed and acetone vehicle was used, these dermal effects are generally consistent with those observed in oral studies of CDFs.

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Based on studies of 2,3,7,8-TCDD and PCBs, there appears to be a common systemic mechanism for many of the dermal and ocular manifestations of CDFs. Chloracne starts with formation of keratin plugs in the pilosebaceous orifices and inflammatory folliculitis. The folliculitis stimulates keratinization of the sebaceous gland ducts and outer root sheath of the hair, leading to formation of keratin cysts (Emmett 1986). The pathology of swollen eyelids is due to keratinization of the Meibomian gland, which is homologous with chloracne.

In conclusion, there is strong human and animal evidence indicating that dermal effects are likely to occur in people exposed to CDFs in the vicinity of hazardous waste sites if levels are high enough.

Other Systemic Effects. Animal studies provide conclusive evidence that oral exposure to CDFs causes a wasting syndrome characterized by progressive decreased weight gain, with immediate moderate to severe body weight loss generally preceding death (Brewster et al. 1988; Kunita et al. 1984; Luster et al. 1979a, 1979b; Moore et al. 1976, 1979; Oishi et al. 1978; Pluess et al. 1988a, 1988b; Poiger et al. 1989). Wasting was observed with 2,3,7,8-substituted tetra-, penta-, and hexaCDF congeners in single dose and intermediate duration oral studies with guinea pigs, rats, and monkeys. Decreased weight gain and weight loss also occurred in mice that were dermally treated with 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF for 20 weeks (Hebert et al. 1990). Structure-activity studies in which CDFs were administered to rats by a single intraperitoneal injection have also demonstrated that the tetra- to hexaCDF congeners, which are fully substituted in the lateral two, three, seven, and eight positions are most active in inhibiting body weight gain (Bandiera et al. 1984b; Holcomb et al. 1988; Mason et al. 1985; Safe et al. 1986). Information on body weight changes has not been reported for Yusho and Yu-Cheng patients (Kuratsune 1989; Rogan 1989), except for decreased birth weights (see Developmental Effects). Animal and human evidence therefore indicates that the wasting syndrome is independent of exposure route. The mechanism of the wasting syndrome has been extensively investigated in animals treated with 2,3,7,8-TCCD but, although basically linked to Ah-receptor binding (see Section 2.3.5), is not clearly understood (ATSDR 1994). Evidence indicates that some other factor(s) than decreased food or water consumption contributes to the weight loss. The mechanism of wasting may be related to effects on thyroid hormones that regulate fat mobilization and utilization of fatty acids in adipose tissue, influence norepinephrine-mediated, nonshivering thermogenesis, or cause anorexia (Rozman et al. 1985; Pazdernik and Rozman 1985; Aust 1984). Appetite suppression due to increased levels of tryptophan in the hypothalamus may also be involved in the wasting syndrome (Rozman et al. 1991; Weber et al. 1991a, 1991b). Tryptophan is

a precursor of the neurotransmitter serotonin. Elevated tryptophan levels, which cause increased serotonin release in the brain, are due to reduced gluconeogenesis, probably resulting from inhibition of phosphoenolpyruvate carboxykinase (a regulatory enzyme in gluconeogenesis). Although the wasting syndrome is a characteristic effect of CDFs in animals usually associated with lethality, it is possible that body weight changes could occur in people exposed to sufficiently high levels.

There is some evidence that the adrenal gland may be a target of CDFs. Studies of Yusho victims showed increased urinary excretion of 17-ketosteroids and 17-hydroxycorticosteroids (Nagai et al. 1971). Single lethal doses of 2,3,7,8-tetraCDF or 2,3,4,7,8-pentaCDF caused adrenal hemorrhage in guinea pigs, but a lethal dosage of 2,3,7,8-tetraCDF in an intermediate duration study produced no consistent change in serum hydrocortisone levels (Luster et al. 1979a, 1979b; Moore et al. 1979). Increased levels of corticosteroids are not good indicators of adrenal toxicity because they can be caused by adrenal stimulation due to stress or diseases. Reduced adrenal function has been observed in animals exposed to 2,3,7,8-TCDD or PCBs, apparently due in part to decreased corticosterone synthesis from decreased adrenal cholesterol side-chain cleavage and 21-hydroxylation of progesterone (ATSDR 1993, 1994; Goldstein and Safe 1989). The available data suggest that the adrenal may be a possible target of CDFs.

Immunological Effects. 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 hypersensitive response, have been observed in Yusho and Yu-Cheng victims (Chang et al. 1981, 1982a, 1982b; Kuratsune 1989; Lu and Wu 1985; Nakanishi et al. 1985; Rogan 1989; Shigematsu et al. 1971). 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 have been consistently observed following oral exposure in all tested species, including offspring of rats exposed during gestation (see Developmental Effects). Histological changes were occasionally reported in spleen (e.g., hypocellularity of lymphoid elements) and lymph nodes (atrophic changes). Immune system tissues other than thymus were not routinely examined because studies with CDDs suggested that the thymus would be a sensitive target organ for CDFs. Single doses ≤3 μg/kg 2,3,4,7,8-pentaCDF and 5 μg/kg/day 2,3,7,8-tetraCDF induced thymic changes in guinea pigs (Moore et al. 1979). The 3 μg/kg/day LOAEL is used as the basis for an acute-duration MRL for oral exposure. This dose also caused reduced muscle mass in guinea pigs (see Musculoskeletal Effects). In intermediate duration oral studies, effects on thymus weight and histology

were induced at dosages ≥0.1 μg/kg/day 2,3,4,7,8-pentaCDF in rats (Pluess et al. 1988a; Poiger et al. 1989), 0.5 μg/kg/day 2,3,7,8-tetraCDF in guinea pigs (Luster et al. 1979a, 1979b), and 2.1 μg/kg/day 2,3,7,8-tetraCDF in monkeys (McNulty et al. 1981). Decreased thymus and spleen weights with atrophy were found in mice dermally exposed to 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF (Hebert et al. 1990).

CDFs substituted in the 2,3,7,8 positions are more immunotoxic than congeners without full lateral substitution and 2,3,4,7,8-pentaCDF appears to be most immunotoxic. For example, 13-week diet studies in mice showed LOAELs of 0.1, 1, 10 and >300 for 2,3,4,7,8-pentaCDF, 1,2,3,6,7,8-hexaCDF, 1,2,3,7,8-pentaCDF, and 1,2,3,4,8-pentaCDF, respectively (Pluess et al. 1988a, 1988b; Poiger et al. 1989). This pattern of toxicity is illustrated by effective doses for decreased splenic response to sheep red blood cells in mice. Mice given a single intraperitoneal injection of 2,3,4,7,8-pentaCDF, 2,3,7,8-tetraCDF, 1,2,3,7,9-pentaCDF, or 1,3,6,8-tetraCDF had estimated ED_{50} values of 1, 4.3, 241.4, and 10,924 μg/kg/day, respectively, for the splenic response (Davis and Safe, 1988). Estimated ED_{50} values for the splenic response in mice administered 10 intraperitoneal doses of heptaCDF congeners in 12 days were 4,499, 4,908, 490,800, and 613,500 μg/kg/day for 1,2,3,4,6,7,8-heptaCDF, 1,2,3,4,7,8,9-heptaCDF, 1,2,3,4,6,7,9-heptaCDF, and 1,2,3,4,6,8,9-heptaCDF, respectively (Dickerson et al. 1990). The same congeneric pattern of activity has also been observed for thymus weight decrease in rats following a single intraperitoneal injection of CDFs (Bandiera et al. 1984b; Mason et al. 1985; Safe et al. 1986). In the only oral study that investigated suppression of the splenic response to sheep red blood eels, the ED_{50} for a single dose of 1,2,3,4,6,7,8-heptaCDF was estimated to be 208 μg/kg in mice (Kerkvliet et al. 1985).

Effects of CDFs on immunocompetence have been evaluated in two intermediate duration oral studies. Macrophage inhibition index and proliferation of lymphocytes following *in vitro* stimulation with a T-lymphocyte mitogen (phytohemagglutinin) decreased in guinea pigs following intermediate duration exposure to 2,3,7,8-tetraCDF (Luster et al. 1979a, 1979b). A study of mortality in mice treated with an uncharacterized mixture of 88% pentaCDFs and 12% tetraCDFs and subsequently challenged with a bacterial endotoxin (*E. coli* lipopolysaccharide) were inconclusive (Oishi and Hiraga 1980). Although these data are only suggestive of functional alterations in immune response, it is likely that altered function is part of the spectrum of immunological effects of CDFs. The immunotoxicity of CDFs, 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.

The lowest dose of 2,3,4,7,8-pentaCDF producing thymic changes in rats (0.1 μg/kg/day) (Pluess et al. 1988a; Poiger et al. 1989) is lower than LOAELs for systemic and nonhepatic effects in other intermediate duration studies. However, the immune system is likely to be more sensitive than the liver. As discussed in Developmental Effects, evidence of thymic toxicity has also been observed in offspring of exposed rats. The animal studies clearly show that immunotoxicity is one of the major and most sensitive effects of CDFs and this is supported by some human data. Therefore, there is a possibility that exposure to CDFs around hazardous waste sites could produce immunological effects in humans.

Neurological Effects. Studies of people exposed during the Yusho and Yu-Cheng incidents showed that various neurological symptoms were common, including numbness, weakness, and neuralgia of limbs and hypesthesia, as well indications of reduced sensory and motor nerve conduction velocities (Chen et al. 1985a; Chia and Chu 1984, 1985; Kuratsune 1989; Kuroiwa et al. 1969; Rogan 1989). No information is available on the mechanism of the reduced nerve conduction velocities (e.g., loss of myelin). There is evidence of delayed neurobehavioral development in children born to mothers with Yu-Cheng exposure (Rogan et al. 1988; Yu et al. 1991). Studies of animals orally treated with CDFs provide no definitive conclusions on possible neurobehavioral toxicity because sensitive neurological tests were not performed. Rats given single lethal doses of 2,3,4,7,8-pentaCDF displayed nonspecific signs of toxicity, including piloerection and splayed and hunched posture. Intermediate duration administration of sublethal dosages of a tetra-, penta-, and hexaCDF mixture caused grossly observable cerebral edema and flabby brain appearance in rats (Brewster et al. 1988; Oishi et al. 1978). Single lethal doses of 2,3,4,7,8-pentaCDF or 2,3,7,8-tetraCDF did not alter brain histology in guinea pigs and mice (Moore et al. 1979). The findings in the animal studies are not indicative of the presence or absence of neurological effects and could be secondary to other changes occurring in intoxicated or

dying animals. There is evidence that CDDs caused peripheral neuropathy and other neurological effects in humans and minor alterations in brain neurotransmitters in animals (ATSDR 1994). The available evidence thus provides some indication that the nervous system is a potential target of CDF toxicity and that some types of neurological effects could occur in populations around waste sites if levels of CDFs were high enough.

Reproductive Effects. Irregular menstrual cycles, abnormal basal body temperature patterns, and decreased urinary excretion of estrogens, pregnanediol, and pregnanetriol were observed in female Yusho patients (Kusuda 1971). These alterations suggested possible corpus luteum insufficiency and retarded follicular maturation. Fertility, fecundity, and rates of spontaneous abortion, however, have not been studied in either Yu-Cheng or Yusho patients (Kuratsune 1989; Rogan 1989). Histology of the ovaries, uterus, and testes was normal in rats orally exposed to 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, 1,2,3,4,8-pentaCDF, or 1,2,3,6,7,8-hexaCDF in an intermediate duration study (Pluess et al. 1988a, 1988b; Poiger et al. 1989). A single 80 µg/kg intraperitoneal dose of 2,3,4,7,8-pentaCDF caused significantly reduced uterine peroxidase activity and uterine wet weight in 25-day-old Sprague-Dawley rats (Astroff and Safe 1990). Antiestrogenic effects of 2,3,7,8-tetraCDF, 2,3,4,7,8-pentaCDF, 6-alkylated-1,2,3-trichlorodibenzofurans, and 2,3,7,8-TCDD in rats, mice, and/or human breast cancer cell lines have also been reported (Astroff and Safe 1988, 1990, 1991; ATSDR 1994; Krishnan and Safe 1993; Zacharewski et al. 1992), and 2,3,7,8-TCDD induces decreased fertility and other reproductive effects in female rodents and monkeys (ATSDR 1994). A single dose study with 2,3,4,7,8-pentaCDF caused no testicular histologic changes in rats (Brewster et al. 1988), although intermediate duration exposure to a mixture of tetra-, penta-, and hexaCDFs decreased seminal vesicle and ventral prostate weights and testicular testosterone concentrations in this species (Oishi et al. 1978). Additionally, a single oral dose of 2,3,4,7,8-pentaCDF or 2,3,7,8-tetraCDF caused hypocellularity of the seminiferous tubules in guinea pigs (Moore et al. 1979). The biological significance of the testicular changes induced by the CDFs is uncertain because reproductive function was not assessed. However, androgenic effects also occurred in rats orally treated with 2,3,7,8-TCDD, including reduced serum testosterone and dihydrotestosterone levels and reduced spermatogenesis (ATSDR 1993). Studies with 2,3,7,8-TCDD suggest that androgenic deficiency may be due to decreased androgen synthesis and that altered testicular morphology may be due to changes in lipid peroxidation (ATSDR 1994; Goldstein and Safe 1989). Although there is no conclusive evidence that CDFs cause reproductive effects in humans, the findings in male animals suggest that effects may

occur. This information is important for populations residing near hazardous waste sites who may be exposed to CDFs for a long period of time.

Developmental Effects. Various signs of toxicity have been observed in children born to mothers exposed during the Yusho and Yu-Cheng incidents (Funatsu et al. 1971; Gladen et al. 1988, 1990; Hsu et al. 1985; Lan et al. 1987; Rogan et al. 1988; Rogan, 1989; Taki et al. 1969; Yamaguchi et al. 1971; Yoshimura 1974; Yu et al. 1991). 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. No exposure-related congenital malformations have been reported in children born to Yusho or Yu-Cheng mothers. 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 maternotoxic and that hydronephrosis is induced at lower doses than cleft palate (Bimbaum 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 1994). The strain of mouse (C57BL/6N) tested in these oral studies is known to be Ah-responsive (Morrissey and Schwetz 1989), and a single intraperitoneal dose of 0.6 mg/kg 2,3,7,8-tetraCDF on day 12 of gestation induced high incidences of cleft palate and hydronephrosis in Ah-responsive inbred mouse strains, but no cleft palates and few fetuses with hydronephrosis in 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 1994; Morrissey and Schwetz 1989) indicate that developmental toxicity of CDFs is mediated by the Ah receptor (see Section 2.3.5). 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 have been shown to 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. Studies of 2,3,4,7,8-pentaCDF in rats have shown induction of hydronephrosis but, no cleft palate, at fetolethal doses, and evidence of thymus toxicity at doses lower than those inducing hydronephrosis in rats or mice (Couture et al. 1989; Masden and Larsden 1989). The lowest doses producing decreased thymus weight occurred in rat offspring examined at age 1 week, and an accompanying cross-fostering experiment showed that *in utero* and lactation exposure contributed almost equally to the effect. This indicates that the immune system is a more sensitive developmental

end point than either hydronephrosis or cleft palate. Considering the reports in humans and strong evidence from animal studies indicating developmental toxicity of CDFs, the possibility that developmental effects may occur in humans exposed to sufficient levels of CDFs around hazardous waste sites cannot be dismissed.

Genotoxic Effects. Limited information was located regarding genotoxic effects of CDFs in humans or animals. As indicated in Section 2.2, the frequency of sister chromatid exchanges increased in subjects exposed orally to CDFs and PCBs compared to control subjects (Lundgren et al. 1988). It should be noted, however, that there was no correlation between serum levels of CDF congeners and increases in sister chromatid exchange frequency. The investigators explained that this lack of correlation was possibly due to the fact that the concentration of PCBs in serum from these individuals was 1,000 times higher than that of CDFs, and to the fact that great daily variations exist in the tissue/blood concentration ratios of CDFs, compared to PCBs.

The mutagenicity of several CDF congeners has been evaluated in microorganisms. In assays with *Salmonella typhimurium* bacteria, octaCDF and 2,3,7,8-tetraCDF were not mutagenic in strains TA98, TA100, TA1535, TA1537, and TA1978 (Schoeny 1982). In addition, octaCDF was not mutagenic in strains TA92, TS24, TA2322, and TA2637. These assays were conducted with and without a metabolic activating system derived from rat liver. Testing of the four monochlorinated dibenzofurans in *S. typhimurium* TA98 and TA100 showed that 3-monoCDF was the only strongly mutagenic congener (Matsumoto et al. 1988). This congener was mutagenic both with and without rat liver metabolic activation preparation, but metabolic activation increased mutagenic potency in both strains. 2-monoCDF was weakly mutagenic in strain TA98 with and without metabolic activation, and l- and 4-monoCDF showed no significant mutagenicity. Further testing with 3-monoCDF in *S. typhimurium* TA98 showed that this congener can be activated by both microsomal and other (cytosolic) enzymes from rat liver (Matsumoto and Ando 1991). 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). Although it appears that CDFs are generally not mutagenic in *in vitro* assays, due to their enzyme induction properties, they may potentiate the genotoxic activity of other compounds by activation to reactive intermediates. Genotoxic effects of other halogenated aromatic hydrocarbons are not known to be Ah receptor mediated.

2. HEALTH EFFECTS

Cancer. There is no conclusive evidence that CDFs are carcinogenic in humans. Human studies involving oral exposure include a retrospective mortality study of Yusho victims (Kuratsune et al. 1987) and an informal analysis of deaths associated with Yu-Cheng exposure (Hsu et al. 1985). Mortality from liver cancer increased significantly in the epidemiology study, but this cannot be definitely attributed to Yusho exposure, due to inconsistent occurrence among study prefectures. Approximately half of the observed Yu-Cheng deaths were attributed to hepatoma, cirrhosis, or unspecified liver diseases, but specific incidences and comparison values were not reported and background prevalences of hepatitis B, cirrhosis, and liver cancer in Taiwan are high.

No studies were located regarding cancer in animals after inhalation or oral exposure to CDFs. Dermal application of 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF for 20 weeks did not induce skin proliferative lesions in mice, but there was no post-treatment observation (Hebert et al. 1990). However, these CDFs as well as 2,3,7,8-tetraCDF promoted development of skin hyperproliferative nodules and squamous cell papillomas in mice following application of the tumor initiator MNNG (Hebert et al. 1990; Poland et al. 1982). Weekly subcutaneous injections of 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF for 4 or 20 weeks promoted development of enzyme-altered hepatic foci (putative preneoplastic lesions) and liver neoplasms in rats following promotion with N-nitrosodiethylamine (Nishizumi and Masuda 1986; Waern et al. 1991). Liver neoplastic nodules, hepatocellular carcinomas, and/or subcutaneous tumors were observed in some rats 104 weeks following subcutaneous injection of 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF as a single dose (61.5-69.6 μg/kg) or four weekly doses (38.1-40 μg/kg/week) (Nishizumi 1989). These findings are difficult to assess due to the small numbers of responders (one to two) and treated animals (five) in each group. Although the human studies are insufficient for evaluating possible carcinogenicity, the animal data suggest a potential for tumor promotion by CDFs.

Results of epidemiological studies and animal testing provide some evidence that 2,3,7,8-TCDD is carcinogenic (ATSDR 1994). The relevance of these findings to CDFs is unclear because it is not known if a common mechanism would be involved.

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to CDFs are discussed in Section 2.5.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by CDFs are discussed in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed

dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, "Populations That Are Unusually Susceptible."

2.5.1 Biomarkers Used to Identify or Quantify Exposure to CDFs

CDFs are pervasive environmental contaminants found in body tissues and fluids of the general population. Because they are lipophilic and have long half-lives, certain CDF congeners containing the 2,3,7,8-chlorine substitution pattern (particularly 2,3,4,7,8-pentaCDF and 1,2,3,4,7,8-hexaCDF) preferentially accumulate in lipid-rich tissues, especially adipose, and are present in whole blood, serum, or plasma and human milk. High amounts of CDFs are also found in the liver, and CDFs have been found at lower concentrations in all other tissues examined to date. Serum and adipose CDF levels are indicators of exposure that may provide an estimate of body burden because, as discussed in Section 2.3.2, some studies have reported that levels of CDFs and congener patterns are similar in serum, adipose, and other tissues when expressed on a fat weight basis (Ryan et al. 1985a; Schecter and Ryan 1989). However, concentrations of CDFs on a fat basis are higher in liver than in adipose (Beck et al. 1990; Thoma et al. 1990). A study of PCB exposure suggests that measurement in both serum and adipose may be more predictive of body burden than each parameter by itself, because concentration in serum varied with the concentration of lipids in serum (Brown and Lawton 1984). Measurements of CDFs in human milk have been used in general monitoring studies and provide some information on previous exposures, no reports were located that used these data to estimate body burden or environmental exposure levels. Quantitative exposure to CDFs can be estimated if the steady-state body burden and elimination half-lives of congeners are known. An elimination half-time from blood of ≈2-2.5 years was estimated for 2,3,4,7,8-pentaCDF and 1,2,3,4,7,8-hexaCDF in Yu-Cheng patients (Ryan et al. 1992b). Sampling was conducted over a g-year period starting 2 years after the incident. The same investigators (Ryan et al. 1992b) calculated a median elimination half-time of 10 years for the same congeners in Yusho patients. In this case, sampling was conducted over an 8-year period, but starting 14 years after the poisoning had occurred. Hair analysis may be a useful method for identifying recent exposure to CDFs in ambient air (Schramm et al. 1992).

Chloracne and changes in the Meibomian glands of the eyelid are effects clearly associated with significant exposure to CDFs based on outcomes of the Yusho and Yu-Cheng incidents. Although chloracne and lesions of the eyelid are biomarkers that are distinct and easily observed, they may not be the most sensitive indicators of human exposure. Additionally, these effects are not associated

specifically with CDFs, as they also can be induced by other chloroaromatic compounds (e.g., CDDs) that seem to act by a common Ah receptor-mediated mechanism (see Section 2.3.5). As discussed in Section 2.5.2, chloracne in Yu-Cheng victims was associated with an estimated body burden of 4.0 μg/kg/day of 2,3,4,7,8-pentaCDF equivalent (PEQ), or about 300 μg (PEQ) in an adult (Ryan et al. 1990).

2.5.2 Biomarkers Used to Characterize Effects Caused by CDFs

Body burden is a biomarker that may be useful for predicting effects of CDFs. A body burden associated with chloracne was calculated using total blood levels of CDFs in symptomatic Yu-Cheng victims (Ryan et al. 1990). An estimated mean uptake associated with chloracne was 4.0 μg/kg/day (PEQ), using a 1:l ratio of the two most toxic congeners (2,3,4,7,8-pentaCDF and 1,2,3,4,7,8-hexaCDF) and a 5:l ratio in relative toxicity of these congeners. This is equivalent to ≈300 μg (PEQ) and 150 μg of 2,3,7,8-TCDD (TEQ) for an adult person. A comparison using 2,3,7,8-TCDD toxicity equivalent factors (see Section 2.4) showed that this estimate is >200 times higher than uptakes estimated from current average total levels of 2,3,7,8-substituted CDFs and CDDs in adipose in normal American and Canadian populations. Levels of CDFs in adipose could also be a useful biomarker for effects. Overall severity of clinical findings in six Yusho patients (four female, two male) was quantified using a numerical rating score and compared to subcutaneous adipose concentrations of 2,3,4,7,8-pentaCDF, 1,2,3,4,7,8-hexaCDF, and 1,2,3,6,7,8-hexaCDF individually and in combination (Nakagawa and Takahashi 1991). For the four females, there was a strong correlation between the total score of clinical findings and adipose concentration of CDFs (r=0.9885 for total CDFs; r=0.8645-0.9804 for individual congeners). The correlation was weaker for the entire group of six patients (r=0.4833 for total CDFs; r=0.4416-0.5291 for individual congeners), the small number of males precluded additional analysis to determine if this was due to gender-related differences in response or the small group size.

Biochemical changes (e.g., increased serum levels of hepatic enzymes, disorders of lipid and carbohydrate metabolism, unbalanced porphyrin metabolism), and/or changes in liver size, ultrastructure, or histology can indicate effects induced by CDFs, but are not specific for these or other chemicals. Biochemical changes in the placenta of women exposed during the Yu-Cheng incident were evaluated for possible use as biomarkers (Lucier et al. 1987, 1990; Sunahara et al. 1987). Decreased placental epidermal growth factor receptor phosphorylation capacity was associated with

decreased birth weights, but this is likely to be a general effect of similarly structured chloroaromatic compounds. The caffeine breath test (CBT) appears to be a sensitive but nonspecific method for characterizing exposure and/or effects of CDFs and related chemicals (Lambert et al. 1992). In this test, [^{13}C-methyl] caffeine is ingested by subjects, and hepatic cytochrome P-450IA2-dependent caffeine 3-N-demethylase activity is monitored by determining the amount of caffeine exhaled as radiolabeled CO,. The CBT is not specific for CDFs since CDDs, PCBs, and other polyaromatic hydrocarbons also induce cytochrome P-450IA. In conclusion, no specific biomarkers of effects of CDFs were identified.

Additional information regarding biomarkers for effects can be found in OTA (1990) and CDC/ATSDR (1990). For a more detailed discussion of the health effects caused by CDFs, please see Section 2.2 of Chapter 2.

2.6 INTERACTIONS WITH OTHER SUBSTANCES

Since concurrent exposure to mixtures of CDFs, CDDs, and other chloroaromatics is common in the general environment, studies regarding interactions of CDFs with other substances have aimed almost exclusively at determining possible changes in the relative potency of individual congeners in the presence of other congeners or 2,3,7,8-TCDD. This is largely because in using the TEF approach to risk assessment of CDFs and CDDs (see Section 2.4), which assumes additivity of toxic responses, it is important to know whether or not interactions between congeners play a role in the final expression of a particular mixture's toxicity. Therefore, it is of vital importance to elucidate whether interactions occur and what is their nature, so that toxicity of mixtures is appropriately estimated, including mixtures associated with hazardous waste sites as well as the Yusho and Yu-Cheng incidents. The validity of the TEF approach for assessing mixtures of CDFs and CDDs has been investigated using both environmental (Eadon et al. 1986) and experimental mixtures (De Vito et al. 1993; Pluess et al. 1988b; Poiger et al. 1989) with varying results depending upon the end point assessed, as discussed below.

The reported guinea pig oral LD_{50} for a soot sample from the Binghamton State Office Building PCB transformer fire, which contained a mixture of CDDs, CDFs, and PCBs, was found equivalent to 58 ppm 2,3,7,8-TCDD (Eadon et al. 1986). The corresponding 2,3,7,8-TCDD equivalents of the soot sample ranged from 2 to 19 ppm based on subchronic toxicity data. Using data from the literature to

estimate the potency of the individual congeners in the soot, the investigators predicted that the soot contained ≈22 ppm 2,3,7,8-TCDD equivalents. This agreement between predicted and observed concentrations was considered good in light of the uncertainties associated with the analytical methods and toxicological data.

Additive effects, as well as usefulness of the TEF approach, have also been demonstrated in long-term feeding studies. Rats were fed a diet containing a mixture of 2,3,7,8-TCDD, 1,2,3,7,8-pentaCDF, and 1,2,3,6,7,8-hexaCDF for 13-weeks (Pluess et al. 1988b; Poiger et al. 1989). This mixture, which contained 1.5 ppb of 2,3,7,8-TCDD equivalents, induced toxic lesions in the thymus and liver of comparable severity to that caused by a dose of 2 ppb of 2,3,7,8-TCDD alone, indicating that the single compounds additively contribute to the toxicity of the mixture as predicted for whole animals.

A more recent study (De Vito et al. 1993) provides some evidence that the TEFs may be inadequate or need reevaluation, although only single response was evaluated. In this study, hepatic, skin, and lung EROD, and hepatic acetanilide-4-hydroxylase activities were determined in mice that were fed presumed equipotent doses (based on published TEFs) of 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, 1,2,3,4,6,7,8,9-octa CDF, 2,3,7,8-TCDD, and several PCB congeners for 4 weeks. It was found that the doses did not produce equivalent induction of enzyme activity for many of these chemicals, indicating that the TEFs do not reliably predict potency at the enzyme level.

Administration of a mixture of 25 nmol 2,3,7,8-TCDD/kg and 200 nmol 2,3,7,8-tetraCDF/kg as a single subcutaneous injection to pregnant mice on days 9-11 of gestation resulted in an incidence of 80% cleft palate in the fetuses examined at day 18 (Krowke 1986). When each chemical, at the same concentrations, were administered separately, the incidence of cleft palate was 34% for 2,3,7,8-TCDD and 40% for 2,3,7,8-tetraCDF, suggesting an additive whole animal response for the mixture. Weber et al. (1985) had previously reported a more adequate analysis of similar results by showing dose additivity (by probit model analysis) between 2,3,7,8-tetraCDF and 2,3,7,8-TCDD regarding cleft palate incidence after oral administration to mice. Also, mixtures of 2,3,4,7,8-pentaCDF and 1,2,3,4,7,8-hexaCDF and of 2,3,4,7,8-pentaCDF and 2,3,4,5,3',4'-hexachlorobiphenyl had additive teratogenic effects (cleft palate and hydronephrosis) when administered orally to pregnant C57BL/6N mice (Birnbaum et al. 1987). Probit analysis of the data revealed parallel dose-response curves, which is compatible with a common and additive mechanism of action for whole animal data.

Co-treatment of DBA/2J mice with single intraperitoneal injections of 200 nmol 2,3,7,8-TCDD/kg and 50, 200, or 800 umol 1,3,6,8-tetraCDF/kg inhibited AHH induction 13%, 39%, and 18%, and EROD induction 17%, 34%, and 21%, respectively, compared to 2,3,7,8-TCDD alone (Bannister and Safe 1987). Therefore, the maximum partial antagonist activity of 1,3,6,8-tetraCDF was obtained at an agonist/antagonist ratio of 1,000/l. In C57BL/6J mice, co-treatment with 15 nmol 2,3,7,8-TCDD/kg and 10, 50, 100, 200, and 500 μmol 1,3,6,8-tetraCDF/kg significantly inhibited both AHH and EROD only at 200 μmol 1,3,6,8-tetraCDF/kg. In this case the maximum partial antagonist activity occurred at an agonist/antagonist ratio of 13,300/l. These results suggest that antagonist activity depends on the strain and the relative concentration ratios of agonist and antagonist.

Administration of single intraperitoneal doses of 1,3,6,8-tetraCDF and 2,3,7,8-TCDD to mice resulted in significant antagonism of the immunotoxic effects of 2,3,7,8-TCDD, as monitored by the splenic plaque-forming cell response to sheep red blood cells (Davis and Safe 1988). Similar results were reported for the combination of 1,3,6,8-tetraCDF and 2,3,4,7,8-pentaCDF. These results are consistent with previously published data showing that 1,3,6,8-tetraCDF has a high affinity for the cytosolic Ah receptor (Keys et al. 1986).

The viability of lymphocytes derived from mice fetal thymus organ culture was reduced by a combination of 3,4,3',4'-tetrachloroazoxybenzene and 2,3,7,8-tetraCDF in an additive manner (Hassoun 1987). While each compound induced a 25-50% reduction in cell viability, an equimolar combination reduced viability by 75%. The results suggest a common mechanism of action for the two chemicals, which is consistent with the fact that both substances bind to the Ah receptor.

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to CDFs than will most persons exposed to the same level of CDFs in the environment. Reasons include genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These parameters result in decreased function of the detoxification and excretory processes (mainly hepatic, renal, and respiratory) or the pre-existing compromised function of target organs (including effects or clearance rates and any resulting end-product metabolites). For these reasons the elderly with declining organ function and the youngest of the population with immature

and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, "Populations With Potentially High Exposure."

No information was located on populations that may be unusually susceptible to CDFs. Strain differences in sensitivity to 2,3,7,8-TCDD toxicity are known to exist in mice and are associated with the Ah receptor (Poland and Glover 1980). Differences in human susceptibility to CDFs could be related to Ah receptor concentration, which has been shown to vary in lymphoid tissue among people (Lorenzen and Okey 1991).

2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to CDFs. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as guide for treatment of exposures to CDFs. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.8.1 Reducing Peak Absorption Following Exposure

No specific information was located regarding the reduction of peak absorption of CDFs in humans. However, long-term medical surveillance (follow-up medical care) strategies have been published (Schecter 1985). After dermal exposure, the affected area should be flushed with plenty of water. In rats, simultaneous oral administration of 2,3,4,7,8-pentaCDF with either liquid paraffin or squalene (2,6,10,15,19,23-hexamethyltetracosane) greatly reduced gastrointestinal absorption of the CDF (Oguri et al. 1987). The relevance of this approach to the treatment of exposed humans is unknown.

2.8.2 Reducing Body Burden

As indicated in Section 2.3, high concentrations of CDF congeners can be retained in the fatty tissues and liver of exposed humans for long periods of time. Retention time is strongly related to the chlorine substitution pattern, and some congeners, particularly those with the 2,3,4,7,8-substitution pattern, may have elimination half-lives from <l year to several years (Schecter and Ryan 1989).

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Since stored CDFs are constantly being redistributed among fatty tissues in the body in accordance with a dynamic equilibrium process among all tissues, it is reasonable to assume that congeners can exert toxic effects in susceptible tissues and organs while retained. One report that examined this issue suggests that fasting may be an effective therapy for reducing signs and symptoms of intoxication with aromatic halogenated hydrocarbons (Imamura and Tung 1984). In this study, 16 individuals who had ingested rice oil contaminated with PCBs and CDFs (Yu-Cheng patients) were maintained on a liquid diet for 7 or 10 days approximately 26 or 35 months after being poisoned. Several months after the fasting period, all the patients showed improvements of signs and symptoms of intoxication. The authors suggested that fasting may stimulate mobilization of CDFs (and PCBs) from adipose tissue to the liver where these chemicals are then metabolized, which would facilitate excretion and reduce body burden. The findings of this study should be interpreted with caution because a control group was not used, small number of subjects were evaluated, the patients volunteered for the study, and some of the end points that were evaluated were subjective. Furthermore, body burden was not monitored. This therapy may not be recommended for pregnant women since, in this case, mobilization of CDFs from adipose tissue into the circulation may translate into increased fetal exposure since transplacental transfer can occur.

In experimental animals, administration of activated charcoal beads, paraffin oil, or squalene after ingestion of 2,3,4,7,8-pentaCDF accelerated (2- to 4-fold) fecal elimination of the compound, mainly by preventing reabsorption from the gastrointestinal tract (Kamimura et al. 1988, 1991; Oguri et al. 1987; Yoshimura et al. 1986). Promotion of fecal excretion of CDFs by cholestryamine, a hypercholesterolemia therapeutic agent used for treatment of poisoning by chlorinated organic agricultural chemicals, was inconclusive in a clinical trial with six Yusho patients in 1989 (Iida et al. 1991; Murai et al. 1991). In this study, fecal excretion of 2,3,7,8-tetraCDF, 2,3,4,7,8-pentaCDF, combined 1,2,3,4,7,8- and 1,2,3,6,7,8-hexaCDFs, 1,2,3,4,6,7,8-heptaCDF, and octaCDF, as well as PCBs, was evaluated throughout the treatment period in which 4 g cholestryamine was ingested three times daily for 6 months.

2.8.3 Interfering with the Mechanism of Action for Toxic Effects

There are no known methods for interfering with the mechanism of action of CDFs. Although the mechanism of action of CDFs is not completely understood, experimental evidence accumulated in recent years indicates that CDFs exert toxic actions by a process involving several steps (Safe 1990a).

This process begins with the binding of CDF congeners to the Ah receptor in the cytoplasm, and this complex leads ultimately to enhancement of the CYPlAl gene expression (see Section 2.3.5). It appears, therefore, that interfering with the initial step, binding to the receptor, or with any of the subsequent steps, would possibly prevent the expression of toxic effects. However, at this time, this concept is purely speculative, mainly because the Ah receptor and its properties, as well as its physiological role, have not been sufficiently characterized.

2.9 ADEQUACY OF THE DATABASE

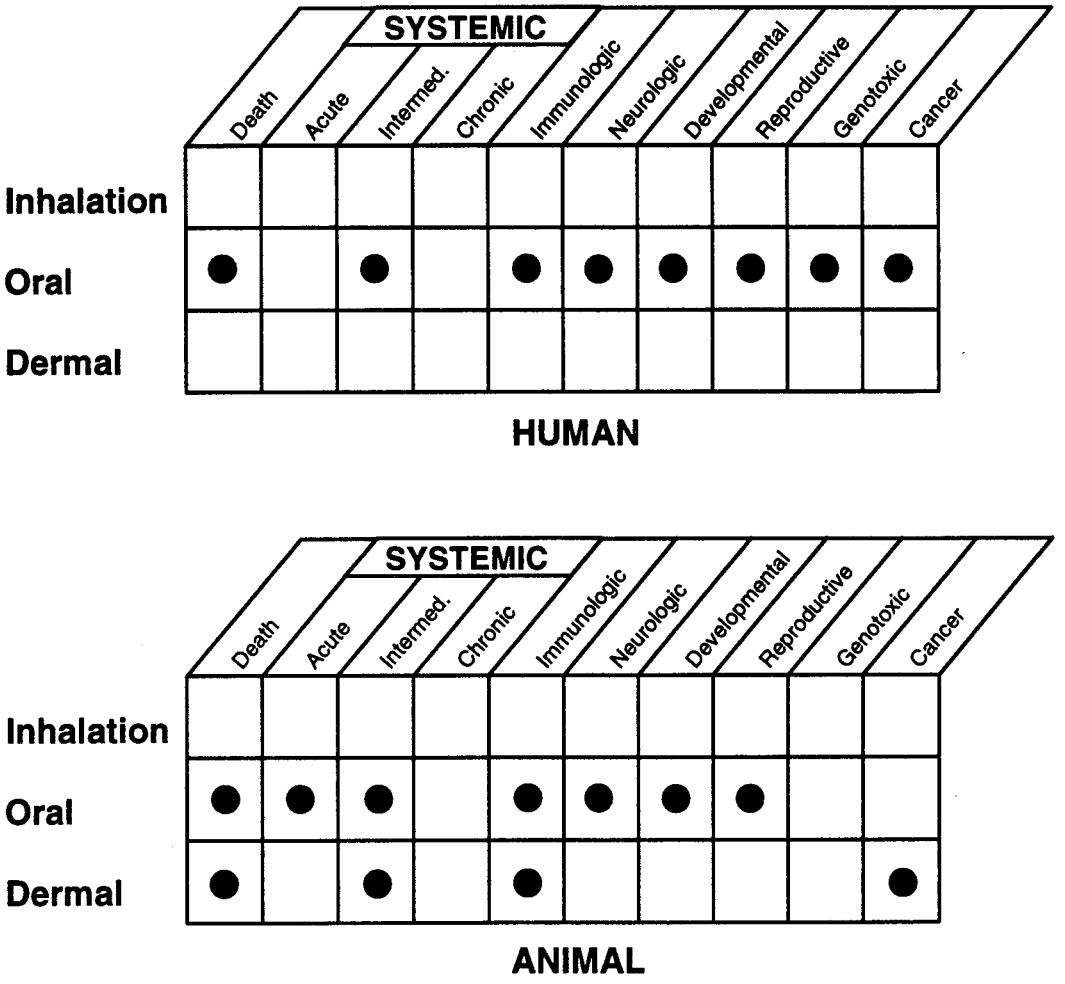
Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of CDFs is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of CDFs.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.9.1 Existing Information on Health Effects of CDFs

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to CDFs are summarized in Figure 2-2. The purpose of this figure is to illustrate the existing information concerning the health effects of CDFs. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as “data needs.” A data need, as defined in ATSDR’s Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

FIGURE 2-2. Existing Information on Health Effects of Chlorinated Dibenzofurans



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As seen in Figure 2-2, information is available regarding death and systemic, immunological, neurological, developmental, reproductive, genotoxic, and carcinogenic effects in humans. Essentially all of the information that pertains to likely effects of CDFs in humans is from the Yusho and Yu-Cheng rice oil poisoning incidents that involved intermediate duration oral exposure to contaminated PCBs. There is strong evidence that CDFs are the main (but not sole) causal agent in the health effects of the Yusho and Yu-Cheng victims. Limited information on effects in humans is available from the Binghamton State Office Building PCB fire incident that involved acute duration exposure to contaminated soot, probably by both dermal and inhalation routes. These studies are not summarized in Figure 2-2 because, as discussed in the introduction to Section 2.4, health effects cannot be attributed specifically to CDFs or any of the other components of the soot due to mixed chemical exposure, possible interactions between CDFs, PCBs, and other components of the contaminated rice oils, lack of confirmed doses, and other confounding factors. Since food is the major source of human exposure in the general population, the oral route is the most likely usual exposure route.

Oral and dermal studies in animals provide data on death, systemic effects resulting from acute- and intermediate-duration exposure, and immunologic, neurologic, developmental, reproductive, and carcinogenic effects. No data were located regarding effects in animals due to inhalation exposure to CDFs.

2.9.2 Identification of Data Needs

Most of the existing information on health effects of CDFs in animals has been obtained in tests using congeners fully substituted in the lateral (2,3,7,8) ring positions, particularly 2,3,7,8-tetraCDF and 2,3,4,7,8-pentaCDF. Studies of 2,3,7,8-tetraCDF were generally performed due to concern for high toxicity based on knowledge of 2,3,7,8-TCDD and other CDDs. Additional testing has shown, however, that CDFs with substitutions in positions 4 and 6 as well as 2,3,7,8 lateral substitutions, particularly 2,3,4,7,8-pentaCDF and 1,2,3,4,7,8-hexaCDF, are also relevant to health effects due to resistance to metabolism and preferential retention of these congeners. As discussed in Section 2.4, the EPA is using the TEF scheme as an alternative interim approach for hazard evaluation of CDFs and CDDs. Since only minimal toxicological data are available for most congeners, additional congener specific studies would provide valuable data for validating the TEF approach. *In vitro* and short-term parenteral injection studies using sensitive end points (e.g., receptor binding affinity,

induction of oxidative enzymes, immunosuppression) have been used for this purpose, but studies using other end points, the oral route, and/or longer durations of exposure would be more informative.

CDFs and the structurally related and more extensively studied CDDs are a concern to ATSDR because of the potential of these chemicals to harm health at relatively low doses. As discussed in Section 2.4, many of the toxic effects of CDDs and CDFs appear to be mediated by a common mechanism, and CDDs frequently occur with CDFs in the environment. Therefore, due to the common mechanism of toxicity, total toxicity of a CDFKDD mixture probably results from the added contribution of both classes of chemicals. Because of this and other issues, including relevant studies of CDDs that are not yet completed, the complex issue of appropriate methodology for quantitatively assessing health risks of CDFs and CDDs is currently being evaluated by ATSDR. Additional information on toxic interactions between CDFs and CDDs, as well as PCBs, would facilitate health risk assessment of CDFs.

Acute-Duration Exposure. Essentially all of the information pertaining to health effects of CDFs in humans is from the Yusho and Yu-Cheng rice oil poisoning incidents. No definite information is available on human health effects of acute oral exposure to CDFs because exposure during these incidents predominately involved intermediate-duration exposure. It is possible that more detailed evaluation of the data on these poisoning incidents could provide insight on possible acute health effects. Information on humans exposed to PCB fires, particularly PCB mixtures not containing chlorinated benzenes (which can form CDDs), could possibly help characterize health effects of CDFs following acute dermal and/or inhalation exposure. Health effects associated with the Binghamton State Office Building electrical transformer fire cannot be attributed solely to CDFs or any of the other components of the soot due to the mixture of chemicals, which included chlorinated benzenes and CDDs, and other confounding factors, as discussed in the Introduction to Section 2.4.

Relatively little information is available on systemic effects of acute duration oral exposure to CDFs in animals. Several effects have been observed, including histopathologic and possible functional changes in the kidneys and gastrointestinal tract and evidence of wasting and anemia (Brewster et al. 1988; Moore et al. 1979). Many of these effects occurred at lethal or other high doses, although effects in the guinea pig, which is the most sensitive species tested in acute oral studies, are relatively well characterized. Since acute toxicity of CDFs may depend more on total dose, rather than frequency of dosing (i.e., after a critical body burden or tissue concentration is reached) (Luster et al.

1979a, 1979b; Moore et al. 1979), and is characteristically delayed in expression, some information from intermediate duration oral studies is relevant to acute exposure. Additional oral studies, however, could provide more suitable information on dose-response relationships at low levels, interspecies and interstrain differences in susceptibility, and effects at doses below the lowest currently known to cause adverse effects (i.e., developmental toxicity) following acute exposure. Data on effects in animals following acute dermal or inhalation exposure to CDFs are not available, although mobilization of CDFs from adipose tissue to target organs is likely to be similar, regardless of the route of exposure. Acute dermal studies are relevant because skin is a route of concern for exposure at or near hazardous waste sites, particularly due to possibilities for brief contact. Acute inhalation studies are unlikely to be relevant, due to the low potential for inhalation exposure in the vicinity of hazardous waste sites and ambient air.

Intermediate-Duration Exposure. Most of the existing toxicity information for CDFs is available from intermediate duration studies of orally-exposed humans following Yusho and Yu-Cheng poisoning and animals. Dermal and ocular effects; mild anemia; mild and transient hepatic alterations, including increased serum levels of triglycerides and liver enzymes and related ultrastructural changes; and bronchitis and other respiratory effects secondary to infection, were most consistently observed in the exposed humans (Kuratsune 1989; Rogan 1989). Although some estimates of doses associated with some effects of Yusho and Yu-Cheng exposure are available, these probably do not reflect the most sensitive toxic end points, as indicated by studies in rats, guinea pigs, and monkeys (Luster et al. 1979a, 1979b; McNulty et al. 1981; Pluess et al. 1988a, 1988b; Poiger et al. 1989). Some systemic effects of intermediate duration oral CDF exposure in animals are consistent with the effects observed in humans, but the animal studies better characterize progression of certain effects (e.g., liver toxicity) and have identified other systemic effects (e.g., wasting syndrome, stomach mucosal lesions) (McNulty et al. 1981; Moore et al. 1979; Oishi et al. 1978; Pluess et al. 1988a; Poiger et al. 1989). Hepatic effects in rats were used as a basis for an intermediate-duration oral MRL. Because of limitations in the database, it is unclear whether different species should be used for studying effects on different target organs. Additional animal studies could help identify NOAELs and characterize other aspects of dose-response for many of the effects, particularly in monkeys, which appear to be more sensitive than guinea pigs and other species based on observations in small numbers of animals (McNulty et al. 1991). These studies could also evaluate effects of oral dosages lower than those currently known to cause adverse effects (i.e., immunotoxicity) in intermediate duration studies, The only information available on systemic toxicity of intermediate duration dermal exposure is from a study in mice, which

found effects in the stomach and liver and on body weight (Hebert et al. 1990). Although these data are suggestive of similar effects by both dermal and oral routes, additional dermal studies could corroborate this and provide information on the sensitivity of other species, and are relevant because the skin is a route of concern for exposure at or near hazardous waste sites. No data were located regarding effects in animals after intermediate-duration inhalation exposure, but inhalation is a minor route of concern for humans.

Chronic-Duration Exposure and Cancer. No information is available on effects in humans or animals following chronic exposure to CDFs by any route. A retrospective mortality study of Yusho victims and an informal survey of Yu-Cheng deaths provides inconclusive evidence of liver cancer (Hsu et al. 1985; Kuratsune et al. 1987). Follow-up and/or more detailed analysis of deaths following Yusho and Yu-Cheng exposure could help ascertain the potential for oral carcinogenicity of CDFs. An intermediate duration study in mice showed no skin neoplastic activity following dermal application of 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF alone, although these congeners as well as 2,3,7,8-tetraCDF promoted development of mouse skin neoplasms (Hebert et al. 1990; Poland et al. 1982). These congeners also promoted development of liver tumors in rats following subcutaneous injection, providing further evidence of tumor promotion by CDFs. Results of a 2-year carcinogenicity study in which 2,3,4,7,8-pentaCDF or 1,2,3,4,7,8-hexaCDF were administered to rats by 1 single or 4 weekly subcutaneous injections are inconclusive due to small numbers of tested animals (Nishizumi 1989). Chronic exposure studies in animals could help elucidate the potential for oral and dermal carcinogenicity of CDFs in the absence of tumor initiators, and provide information on noncancer effect levels. Chronic oral studies should provide information relevant to dermal exposure, because toxicokinetic data suggest that the potential for systemic toxic and carcinogenic effects is likely to be qualitatively similar across routes. Also, chronic low-dose studies in animals would mimic the steady state of lifetime exposure in humans.

Genotoxicity. Limited information is available regarding genotoxic effects of CDFs in humans. Examination of lymphocytes of Yu-Cheng individuals revealed an increased frequency of sister chromatid exchanges. This effect could be attributed to PCBs that were found in the serum of these subjects at a concentration level 1,000 times higher than CDFs (Lundgren et al. 1988), because genotoxic effects of halogenated aromatic hydrocarbons are not known to be Ah receptor-mediated. Only 2,3,7,8-tetraCDF has been tested for genotoxicity in eukaryotic organisms (*S. cerevisiae* yeast) (Fahrig et al. 1978), and only monoCDFs, octaCDF, and 2,3,7,8-tetraCDF have been tested in

prokaryotes (*S. typhimurium* bacteria) (Matsumoto and Ando 1991; Matsumoto et al. 1988; Schoeny 1982). The results of these studies showed that 2,3,7,8-CDF was not mutagenic in *S. cerevisiae* and that CDFs were generally not mutagenic in various strains of *S. typhimurium,* with only 2- and 3-monoCDF inducing some activity. Additional studies with other congeners, in both eukaryotic and prokaryotic organisms *in vitro,* including bacteria, yeast, and rodent and human cells in culture, would provide valuable information regarding structure-activity relationships. Also, cytogenic analysis of human populations exposed to CDFs in occupational settings, or by consumption of food contaminated with CDFs would provide an opportunity to assess the genotoxic potential of these compounds in humans.

Reproductive Toxicity. Irregular menstrual cycles, abnormal basal body temperature patterns, and decreased urinary excretion of estrogens and pregnanediol were observed in female Yu-Cheng patients (Kusuda 1971). Although possibly suggestive of corpus luteum insufficiency and retarded follicular maturation, studies of fertility, fecundity, and rates of spontaneous abortion in Yu-Cheng and/or Yusho would provide more definite information on reproductive toxicity of CDFs. Some intermediate duration oral studies showed no histological alterations in the ovaries, uterus, or testes of rats treated with various CDFs (Pluess et al. 1988a, 1988b; Poiger et al. 1989), although there is some evidence from other oral studies (intermediate duration in rats and acute duration in guinea pigs) that the testes are a target (Moore et al. 1979; Oishi et al. 1978). Although pathological examinations performed as part of 90-day oral toxicity studies would be useful for identifying and corroborating susceptibility of the reproductive system and determining sensitive species, studies assessing effects of CDFs on reproductive function in males and females would be more informative. Such studies would enable the NOAEL region for reproductive effects to be better defined and provide assurance that MRLs are sufficiently protective. No information is available on reproductive effects of CDFs in animals or humans following dermal or inhalation exposure, but limited available toxicokinetic data suggest that the potential for reproductive toxicity is likely to be qualitatively similar across routes (Birnbaum et al. 1980; Brewster et al. 1989).

Developmental Toxicity. Various toxic effects have been observed in children born to mothers exposed during the Yusho and Yu-Cheng incidents, including dermal lesions, decreased birth weights, neurobehavioral deficits, and some perinatal deaths (Furatsu et al. 1971; Gladen et al. 1990; Hsu et al. 1985; Lan et al. 1987; Rogan et al. 1988; Taki et al. 1969; Yamaguchi et al. 1971; Yu et al. 1991). Although no exposure-related congenital malformations have been reported in these children, oral

studies in mice and rats have documented induction of hydronephrosis and/or cleft palate by 2,3,7,8-substituted tetra-, penta-, and hexaCDF congeners (Birnbaum et al. 1987a; Couture et al. 1989; Masden and Larsen 1989; Weber et al. 1984, 1985). Tissues other than kidney and palate were examined only in the rat studies, which provide some evidence indicating that rats are more susceptible to CDFs than mice and that neonatal thymic toxicity is a more sensitive developmental end point than fetal mortality or cleft palate in rats (Couture et al. 1989; Masden and Larsen 1989). Additional studies could verify that thymic toxicity is the most sensitive end point and the rat is the most sensitive species for developmental effects. Immunological evaluations of offspring would be valuable to determine the importance of thymic changes, and neurobehavioral evaluations in monkey offspring would be particularly relevant, due to the deficits observed in children of Yu-Cheng mothers. Since nursing can significantly contribute to offspring body burden and CDFs are retained in adipose long after external exposure has been discontinued, follow-up evaluations of sensitive developmental end points is desirable.

Immunotoxicity. Clinical observations of increased susceptibility to respiratory and dermal infections and various changes in immune parameters in Yusho and Yu-Cheng victims provide limited information on immunological effects of CDFs in humans (Chang et al. 1981, 1982a, 1982b; Kuratsune 1989; Lu and Wu 1985; Nakanishi et al. 1985; Rogan 1989; Shigematsu et al. 1971). Acute and intermediate duration oral exposure to CDFs induces decreased organ weight and atrophy in the thymus and to a lesser extent spleen in rodents and monkeys (Brewster et al. 1988; Luster et al. 1978a, 1979b; McNulty et al. 1981; Moore et al. 1979; Pluess et al. 1988a; Poiger et al. 1989). The induction of thymic toxicity at doses as low or lower than those know to cause other adverse effects in acute- and intermediate-duration studies indicates that the immune system may be one of the most sensitive targets for CDFs and provide a basis for an acute oral MRL. There is suggestive evidence of CDF-induced impaired functional immune response in guinea pigs (Luster et al. 1979a, 1979b), but an immunocompetence test in mice was inconclusive (Oishi and Hiraga 1980). Additional studies would be necessary to determine if the immune system is a critical target of CDFs. Decreased thymus and spleen weights with atrophy also occurred in mice dermally treated with CDFs in an intermediate duration study, indicating that immunological effects of CDFs are unlikely to be route specific (Hebert et al. 1990). No studies were located regarding developmental effects in humans or animals after inhalation exposure to CDFs.

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Neurotoxicity. Various neurological symptoms were common and there were some indications of reduced sensory and motor nerve conduction velocities in Yusho and Yu-Cheng victims (Chen et al. 1985a; Chia and Chu 1984, 1985; Kuratsune 1989; Rogan 1989). Furthermore, it should be mentioned that the developing nervous system appears to be a target for CDFs in children born to mothers with Yu-Cheng exposure (see Developmental Effects). Studies of rats and guinea pigs orally treated with CDFs for acute or intermediate durations provide no definite information on possible neurobehavioral toxicity, because nonspecific and/or insensitive end points (e.g., toxic signs, brain pathology) were evaluated (Brewster et al. 1988; Moore et al. 1979; Oishi et al. 1978). A battery of neurobehavioral studies, including neurohistology, would provide information on the susceptibility of animal species and could be used to corroborate the human data. Follow-up evaluations of the human populations already studied would help determine whether or not deficits observed in infants exposed *in utero* and through nursing progress into more severe alterations. Pertinent information is lacking on neurological effects of CDFs in humans and animals following dermal or inhalation exposure, but existing toxicokinetic data that do not suggest route-specific target organs.

Epidemiological and Human Dosimetry Studies. Studies of the Yusho and Yu-Cheng populations provide a wealth of information on health effects attributable to CDFs, and these populations are the best existing basis for assessing the effects of CDFs (Hsu et al. 1985; Kuratsune 1989; Kuratsune et al. 1987; Rogan 1989) in humans further. Additional studies could possibly provide information on dose-response for sensitive effects and discern which effects represent delayed and/or irreversible toxicity. Follow-up studies would also be useful for more adequately assessing risk of cancer. Municipal incineration workers (Schecter et al. 1991) and certain other worker populations (see Section 5.2.1) may be exposed to CDFs by inhalation and dermally, but co-exposure to CDDs and other chemicals is more of an issue than in the Yusho and Yu-Cheng incidents.

Biomarkers of Exposure and Effect

Exposure. Due to their lipophilicity, CDFs are stored in highest concentrations in adipose tissue and are frequently measured in blood and human milk, and have been found at lower concentrations in all other tissues examined to date. Several studies indicate that serum and adipose levels of CDFs are biomarkers of exposure feasible for estimating body burden or exposure (Brown and Lawton 1984; Ryan et al. 1985a; Schecter and Ryan 1989). Further studies on the predictive value of CDF levels in

human serum, adipose, and milk could provide valuable information that could lead to early detection of exposure.

Effect. A body burden association with chloracne has been calculated for CDFs using data from Yu-Cheng victims (Ryan et al. 1990). Additional studies could evaluate the feasibility of using body burden as a biomarker for predicting other effects of CDFs. Chloracne and many other effects of CDFs, however, are common to other chloroaromatics acting by the Ah receptor-mediated mechanism. There are no specific clinical or biochemical biomarkers of effects for CDFs, although some (e.g., changes in lipid and porphyrin metabolism) may be limited to chloroaromatics acting by the common mechanism. Further studies to identify specific biomarkers of effects of PCBs would facilitate medical surveillance leading to early detection of potentially adverse health effects and possible treatment.

Absorption, Distribution, Metabolism, and Excretion. There are no quantitative data regarding absorption in humans by the inhalation, oral, or dermal routes, but data from accidentally exposed individuals suggest that exposure by any of these routes, or a combination of them, may lead to considerable accumulation of CDFs in tissues (Chen et al. 1985b; Masuda et al. 1985; Schecter and Ryan 1989). The animal data indicate that CDFs (mostly tetra- and pentaCDFs) are efficiently absorbed by the oral route (Birnbaum et al. 1980; Brewster and Birnbaum 1987; Van den Berg et al. 1989). Inhalation absorption data are not available. Dermal absorption data were limited to one study in rats that showed relatively low absorption for two pentaCDFs, compared with oral rates (Brewster et al. 1989). No studies were located in which a range of doses of different CDF congeners were administered by the inhalation, oral, and dermal routes, and for various exposure periods.

As with absorption, distribution data in humans are limited to qualitative information derived from cases of accidental ingestion of food contaminated with CDFs (Chen et al. 1985b; Masuda et al. 1985), cases of occupational exposure through inhalation or dermal contact with CDFs (Schecter and Ryan 1989), and autopsy reports from the general population (Ryan et al. 1985a; Schecter et al. 1989a). These data suggest that CDFs distribute preferentially to tissues with high fat content regardless of the route of exposure. Data derived from oral and dermal administration of single CDF congeners to animals indicate that CDFs distribute first to the liver and are subsequently translocated to adipose tissue for storage (Brewster and Birnbaum 1987; Birnbaum et al. 1980; Brewster et al. 1989; Decad et al. 1981a). Studies regarding distribution through the placenta after inhalation and dermal exposures were not available.

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Data regarding biotransformation of CDFs in humans are limited to individuals who accidentally consumed food contaminated with CDFs (Chen et al. 1985b; Masuda et al. 1985). The use of human cell systems in culture might be considered a useful addition to whole animal studies for studying the metabolic fate of CDFs. The metabolism of some CDF congeners after acute oral administration to rats has been studied (Poiger and Pluess 1989). Although information regarding metabolism following inhalation or dermal exposure is lacking, there is no reason to believe that other pathways would operate after exposure by these routes.

Studies regarding urinary or fecal excretion of CDFs in humans were not located; however, elimination of CDFs through maternal milk is well documented (Van den Berg et al. 1986). Fecal excretion is the main route of elimination of CDFs in animals after acute oral exposure (Birnbaum et al. 1980; Brewster and Birnbaum 1987; Decad et al. 1981a; Weber and Birnbaum 1985). Excretion data following dermal exposure support the oral data, but the information is derived from a single study (Brewster et al. 1989). Although data regarding excretion after inhalation were not located, there is no reason to suspect different patterns of excretion.

Comparative Toxicokinetics. The existing evidence suggests that qualitative differences in the toxicokinetic disposition of CDFs exist among humans and among animal species. However, these differences appear to be highly dependent on the specific congener studied. In general, all species absorb CDFs efficiently and accumulate CDFs in tissues rich in fat. Once absorbed, CDFs distribute in a similar manner in all examined animal species (high initial concentration in blood, liver, and muscle, followed by gradual increase in CDF concentration in adipose tissue) (Bimbaum et al. 1980; Brewster and Birnbaum 1987; Decad et al. 1981a; Weber and Birnbaum 1985). Identification of metabolites in humans and rats suggests that both species share some common biochemical reactions (Chen et al. 1985b; Poiger and Pluess 1989). Experimental data in animals indicate that fecal elimination is the main route of excretion (Bimbaum et al. 1980; Brewster and Bimbaum 1987; Decad et al. 1981a; Weber and Bimbaum 1985), but no human information was located in the existing literature. Analysis of the excreta of humans accidentally exposed to CDFs or living near hazardous waste sites would provide information regarding biotransformation and elimination kinetics in humans. In addition similar target organs have been identified across animal species. Monkeys seem to be one of the most sensitive species tested. Although the toxicological data in humans are limited, adverse cutaneous and ocular (e.g., Meibomian gland) reactions documented in humans (Kuratsune 1989) are

also seen in monkeys (McNulty et al. 1981), suggesting that monkeys may represent a suitable animal model.

Methods for Reducing Toxic Effects. The mechanism by which CDFs enter the blood stream in humans is not known, consequently, there are no established methods for reducing absorption. In rats, however, simultaneous oral administration of paraffin oil or squalene reduced gastrointestinal absorption in an acute-duration study (Oguri et al. 1987). Identification of additional substances that could prevent or delay absorption and that do not represent a toxic risk per se would be valuable. There are no established methods for reducing body burden in humans, but a report indicated that fasting may be effective (Imamura and Tung 1984). Studies examining the effect of fasting in animals exposed to CDFs would provide useful information that can be used to better characterize the effectiveness of this approach. The mechanism of toxic action of CDFs is not completely understood and no methods exist to block the toxic response due to exposure to CDFs. A more complete characterization of the cytosolic receptor protein, to which CDFs are thought to bind, and understanding of physiological effects of receptor blockage would be useful for the possible identification of blockers of that reaction. There are no established methods for mitigation of health effects resulting from exposure to CDFs.

2.9.3 On-going Studies

Dr. S. Safe and his colleagues at the Texas A & M University are studying the antiestrogenic responses in rodents and human breast cancer cells in culture of a series of 1,3,6&substituted CDFs (FEDRIP 1992, 1993). The project involves characterization of the antiestrogenic response in human cells, determination of the mechanism of action of these chemicals, and determination of anti-tumorigenic effects of these compounds in nude athymic mice.

Dr. Safe and his group are also attempting to characterize the structure-activity relationship for higher chlorinated CDFs using a battery of Ah receptor-mediated *in vivo* assays. Furthermore, they also propose to validate the utility of *in vitro* assays for monoxygenase enzyme and cytochrome P-450 mRNA induction as methods for quantitatively assessing the toxic equivalents of complex mixtures. Additional studies conducted by Dr. Safe's group are focusing on characterizing some of the properties of the Ah receptor with newly synthesized radiolabeled CDFs.

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Dr. K. Randerath, also from Texas A & M University, and co-workers are investigating the mechanism of carcinogenesis of halogenated aromatic hydrocarbons by utilizing the highly sensitive 32P-postlabeling assay for detecting DNA adducts (FEDRIP 1992).

Dr. A. Poland and his research team, at the University of Wisconsin, propose to screen human lymphoblastoid cell lines for variants of the Ah receptor and determine the functional significance of these variants. Dr. Poland is also investigating the occurrence of the Ah receptor in invertebrates (FEDRIP 1992).

A group of investigators at SUNY, Albany, headed by Dr. D. Carpenter, are conducting an epidemiological study in a population adjacent to a Superfund-designated landfill contaminated with CDFs and PCBs (FEDRIP 1992). They will try to correlate contaminant levels in fish and wildlife consumed by pregnant and nursing mothers with levels in body fluids and breast milk, and with levels in urine and feces of infants. Studies in rats aim to assess developmental, neurological, and hepatic effects after pre- and postnatal exposure to CDFs. Dr. Carpenter is also studying the effects of CDF exposure on the invertebrate sea snail, *Aplysia.*

Dr. G. Lucier at the National Institute of Environmental Health Sciences (NIEHS) is examining placentas from humans exposed to CDFs in Taiwan and comparing biochemical changes such as enzyme induction in the placentas to those occurring in rats (FEDRIP 1992).

Dr. L. Birnbaum and colleagues at NIEHS are continuing studies designed to elucidate the absorption rates and disposition of CDFs, and the effects of age on these parameters, in experimental animals (FEDRIP 1993).