CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

- Quantitative information regarding absorption of inhaled CDFs in humans and animals was not located; however, CDFs were detected in blood and adipose tissue following inhalation exposures in humans. Absorption of ingested CDFs was estimated to be >90% in adults and nursing infants. Studies conducted in animals demonstrate that CDFs can be absorbed through the skin.

- CDFs are lipid soluble and tend to accumulate in tissue lipid. Tissues that accumulate the highest concentrations of CDFs are adipose and liver. Accumulation of CDFs in liver is facilitated by an inducible binding protein, CYP1A2.

- CDFs are metabolized by the inducible CYP450 enzyme system. Factors that influence metabolism (e.g., chlorine substitution, animal species differences) contribute to variability in toxic potencies of CDFs.

- The major pathways of excretion of absorbed CDFs are feces and urine. Studies conducted in monkeys, mice, and rats indicate that feces are the dominant pathway for excretion of absorbed CDFs.

3.1.1 Absorption

Absorption of Inhaled CDFs. Quantitative information regarding absorption of inhaled CDFs in humans and animals were not located. However, absorption of CDFs can be inferred from detection of CDFs in blood and tissues following accidental or occupational exposure to airborne CDFs (Schecter and Ryan 1989; Schecter et al. 1991a). Subjects were exposed to soot or dust containing CDFs during cleanup operations following a PCB transformer fire or associated with municipal solid waste incineration. The relative contribution of the inhalation, dermal, and oral routes of absorption in these individuals cannot be determined.

Absorption of Ingested CDFs. Several human fecal mass balance studies estimated absorption of dietary CDFs based on short-term measurements of the difference between dietary intake and fecal excretion of CDFs (Abraham et al. 1994; Dahl et al. 1995; Jödicke et al. 1992; Körner et al. 1993; McLachlan 1993; Moser and McLachlan 2001; Pluim et al. 1993; Schlummer et al. 1998; Schrey et al. 1998). These studies cannot distinguish fecal excretion of absorbed CDFs from excretion of unabsorbed CDFs and, as a result, these studies can only estimate net absorption. Studies conducted in monkeys and rodents demonstrate that fecal excretion is a major pathway of excretion for absorbed CDFs; therefore, fecal balance studies are likely to underestimate the absorbed fraction of the ingested dose. In some human studies, fecal

excretion has been found to exceed measured dietary intakes of CDFs (negative balance) suggesting that at least some of the fecal CDF was derived from body stores (Moser and McLachlan 2001; Schrey et al. 1998). Net absorption was found to correlate with concentrations of CDFs in blood lipid, which also suggests a relationship between fecal excretion and body burden (Schlummer et al. 1998). Based on these human studies, net absorption of tetraCDFs, pentaCDFs, and hexaCDFs was estimated to be >90% in adults.

Several fecal mass balance studies evaluated the absorption of CDFs from breast milk; each study evaluated a small number of infants (n=1–4). Based on the amounts of CDFs in the feces, the studies estimated that at least 90% of the lower chlorinated CDFs (tetraCDF, pentaCDF, and hexaCDF) in breast milk was absorbed (Abraham et al. 1994; Dahl et al. 1995; Jödicke et al. 1992; Körner et al. 1993; McLachlan 1993; Pluim et al. 1993). The highly chlorinated CDFs (e.g., heptaCDFs and octaCDF) had the highest excretion rates, likely indicative of lower absorption efficiencies (Abraham et al. 1994; Dahl et al. 1995; Jödicke et al. 1992; Körner et al. 1993). One study of a 5-month-old infant reported that the percentage of ingested CDFs in the stool ranged from 2 to 10% for 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, 1,2,3,6,7,8-hexaCDF, and 2,3,4,6,7,8-hexaCDF and from 40 to 100% for 1,2,3,4,6,7,8-heptaCDF and octaCDF (Abraham et al. 1994). Another study reported estimated absorption of 97–100% for 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, and 2,3,4,7,8-pentaCDF; 88–100% for 1,2,3,4,7,8-hexaCDF and 1,2,3,6,7,8-hexaCDF; and 59–82% for 1,2,3,4,6,7,8-heptaCDF (Dahl et al. 1995).

Studies conducted in rodents have shown that guinea pigs and rats absorbed >80% of the ingested CDF dose (Birnbaum et al. 1980; Brewster and Birnbaum 1987; Decad et al. 1981a; van den Berg et al. 1989). 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 was not dose-related over the dose range tested (Brewster and Birnbaum 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).

Relative bioavailability of CDF congeners in soil was estimated in rats and swine (Budinsky et al. 2008; Finley et al. 2009; Wittsiepe et al. 2007a). Relative bioavailability was measured as the ratio of tissue congener levels following oral dosing with the congener in soil or in a reference vehicle (soil/reference), typically corn oil or some other lipid. These studies showed that the relative bioavailability of CDF congeners was <100% in rats and swine and varied across soil compositions. For example, in rats, the relative bioavailability of 2,3,7,8-tetraCDF in five different soils ranged from 27 to 89% (Budinsky et al. 2008; Finley et al. 2009). Relative bioavailability also varied with congener chlorination; increasing with increasing chlorine content in swine and decreasing with increasing chlorine content in rats (EPA 2010).

The limited data regarding oral absorption of CDFs in animals suggest that, in general, these compounds are absorbed and 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).

Dermal Absorption of CDFs. 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 detection of CDFs in blood and tissues following 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 such cases, the relative contribution of the inhalation, ingestion, or dermal routes cannot be determined.

Limited information is available regarding dermal absorption of CDFs in animals. In a dermal absorption study in male Fischer 344 rats, ^{3}H-1,2,3,7,8-pentaCDF, ^{14}C-2,3,4,7,8-pentaCDF, and ^{14}C-2,3,7,8-tetra-CDF in acetone were applied to the clipped back skin at several dose levels (Brewster et al. 1989). At doses of 34 µg/kg, 25, 34, and 49% of 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, and 2,3,7,8-tetraCDF, respectively, were absorbed over a 3-day period. 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, about 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 (Brewster et al. 1989). In another study of 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 of the animal (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 10-week-old rats was greater than that observed in 36- and 120-week-old animals. A subsequent study by this group reported that approximately 37% of the 34 µg/kg administered dose of 1,2,3,7,8-pentaCDF was absorbed through the skin (Jackson et al. 1993).

The available information indicates that over a 3-day period, the percentage of the dermal dose absorbed for tetra- and pentaCDFs in animals is less than or equal to half of the percentage observed for oral absorption.

3.1.2 Distribution

Tissue Distribution in Humans. Absorbed CDFs tend to distribute and accumulate in tissue lipid because of their relatively high lipid solubility (octanol/water partition coefficients ranging from 10^7 to 10^8) (Jackson et al. 1993; Maruyama et al. 2002). Studies of postmortem concentrations of CDFs have found CDFs in a variety of tissues including blood, bile, adipose, kidney, liver, pancreas, skeletal muscle, and spleen (Bajanowski et al. 2002; Iida et al. 2007; Maruyama et al. 2002; Ryan et al. 1985a, 1986; Schecter et al. 1989a; Watanabe et al. 2013). On a whole weight basis, adipose tissue had the highest concentrations of CDFs, followed by liver, muscle, and kidney (Ryan et al. 1985a). The most prevalent CDFs were 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 (Iida et al. 2007; Ryan et al. 1985a). When the results were expressed on a lipid basis, the concentration of total CDFs was at least twice as great in liver compared to other tissues, consistent with most of the tissue CDFs associated with tissue lipid content (Iida et al. 2007; Ryan et al. 1985a).

Distribution of CDFs in humans has been studied in deceased patients 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). In general, the congeners identified in the tissue and blood of Yusho patients consisted of elevated levels of 2,3,4,7,8-pentaCDF, 1,2,3,4,7,8-hexaCDF,

and 1,2,3,6,7,8-hexaCDF, the most prevalent of which was 2,3,4,7,8-pentaCDF (Ryan et al. 1987a). 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 3.1.3). 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 1 or >1 order of magnitude lower than in the liver (Chen et al. 1985a). 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.

Maternal-Fetal-Infant Transfer in Humans. Maternal fetal transfer of CDFs occurs based on detection of CDFs in tissues and correlations between maternal blood concentrations and concentrations of CDFs in cord blood, the placenta, and fetal tissue (Abraham et al. 1996, 1998; Schecter et al. 1998; Tsukimori et al. 2013; Wang et al. 2002, 2004). Concentrations of CDFs in maternal blood and breast milk were correlated (Boda et al. 2018; Todaka et al. 2010; Tsukimori et al. 2011). The rate of transfer to breast milk was sufficient to decrease the blood maternal CDF body burden and increase the blood elimination half-time (Abraham et al. 1998; Milbrath et al. 2009; Schecter et al. 1996; Wittsiepe et al. 2007b). Breast milk concentrations of CDFs decline during nursing as a result of the decline in maternal CDF stores (Beck et al. 1994; Vigh et al. 2013); see Section 5.6 for breastmilk monitoring data. Intake-fecal mass balance studies conducted on nursing infants found that CDFs ingested by nursing infants are absorbed to varying degrees depending, in part, on degree of chlorination (Abraham et al. 1994, 1996; Jödicke et al. 1992; Körner et al. 1993; McLachlan 1993; Pluim et al. 1993). Absorption of hepta- and octaCDFs were estimated to range from 80 to 90%, whereas absorption of less chlorinated CDFs was >90%.

CDFs were reported in the liver and adipose tissue of a breastfed infant born to a mother in the Yu-Cheng cohort (Masuda et al. 1985). Beck et al. (1990a) detected CDFs in the brain, adipose tissue, thymus, spleen, and liver of three infants who died of sudden infant death syndrome (SIDS) before reaching 1 year of age. Maternal exposures were not reported. Of the three infants, only one had been breastfed 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).

Tissue Distribution in Laboratory Animal Models

Distribution in Monkeys. In rhesus monkeys, following a single intravenous dose of 30.7 µg ^{14}C-labeled 2,3,7,8-tetraCDF/kg, the ^{14}C was rapidly cleared from the blood (Birnbaum et al. 1981). A two-component exponential elimination from blood was observed, with half-times of 1.5 minutes and 1 hour, respectively. Terminal components of the removal of ^{14}C from the blood were not determined in the study. After 21 days, <10% of the ^{14}C remained in the body. When the concentration of ^{14}C was expressed as CDF 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 ^{14}C 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 ^{14}C remaining in blood at day 21 seemed to correspond to metabolites. In marmoset monkeys subcutaneously administered a mixture of CDDs and CDFs, elimination half-times from adipose tissue and hepatic tissue increased with the degree of chlorination (Neubert et al. 1990). The location of the chlorines also influenced the elimination half-times with 2,3,7,8-substituted congeners having longer half-times. The half-times for the CDF congeners tested are presented in Table 3-1.

Table 3-1. Half-Times of Various CDF Congeners in Hepatic and Adipose Tissues of Marmoset Monkeys Subcutaneously Administered a Single Dose of a CDD/CDF Mixture

	Half-times (weeks)	
Congener	Hepatic tissue	Adipose tissue
2,3,7,8-TetraCDF	<0.87 (6 days)	1.39
1,2,3,7,8-/1,2,3,4,8-PentaCDF	0.93 (6.5 days)	1.46
2,3,4,7,8-PentaCDF	8.8	12.3
1,2,3,4,7,8-/1,2,3,4,7,9-HexaCDF	23	68
1,2,3,6,7,8-HexaCDF	14.3	24
1,2,3,7,8,9-HexaCDF	8.2	Not calculated
2,3,4,6,7,8-HexaCDF	18.6	38
1,2,3,4,6,7,8-HeptaCDF	37	Apparently infinite

Table 3-1. Half-Times of Various CDF Congeners in Hepatic and Adipose Tissues of Marmoset Monkeys Subcutaneously Administered a Single Dose of a CDD/CDF Mixture

	Half-times (weeks)	
Congener	Hepatic tissue	Adipose tissue
1,2,3,4,7,8,9-HeptaCDF	79	660
OctaCDF	174	Apparently infinite

CDD = chlorodibenzo-*p*-dioxin; CDF = chlorodibenzofuran

Source: Neubert et al. 1990

A study in marmoset monkeys compared tissue concentrations of CDF congeners in maternal and offspring tissues (Hagenmaier et al. 1990). The concentrations of pentaCDF, hexaCDF, heptaCDF, and octaCDF congeners were considerably lower in fetal (pooled sample from 18-week twins) liver tissue compared to maternal liver tissue. For example, fetal concentrations of 2,3,4,7,8-pentaCDF, 1,2,3,6,7,8-hexaCDF, and 1,2,3,4,6,7,8-heptaCDF were approximately 30, 300, and 700 times, respectively, lower than maternal concentrations. At birth, CDF concentrations in the liver were much lower than adults exposed for a similar duration; the sum of CDF congener concentrations were 1,059 pg/g wet weight in the newborns compared to 54,584 pg/g wet weight in adults. However, the concentration of CDF congeners in adipose tissue were similar in the newborn and adult monkeys. On PND 33, the sum of CDF congener concentrations in infants was approximately 4 times lower than in adults (20,266 versus 87,929 pg/g wet weight). The largest differences were found for the higher chlorinated congeners; the ratios of infant to mother were 0.1, 0.2, and 0.7 for octaCDF, heptaCDF, and hexaCDF, respectively. In contrast, the tetraCDF and pentaCDF congener concentrations in the liver were similar in the infants and adults (ratios of 1.1 and 1.0, respectively) (Hagenmaier et al. 1990).

Distribution in Mice. Distribution of absorbed CDFs has been extensively studied in mice (Aozasa et al. 1995; Decad et al. 1981b; De Jongh et al. 1993; DeVito et al. 1995, 1997, 1998; Diliberto et al. 1999; van Ede et al. 2013; Weber and Birnbaum 1985). Following a single intravenous dose of 30.6 µg ^{14}C-labeled 2,3,7,8-tetraCDF/kg, ^{14}C was concentrated in the liver, adipose tissue, skin, and muscle of C57BL/6J and DBA/2J male mice; these tissues accounted for >75% of the injected dose (Decad et al. 1981b). At all times over a 10-day period (except at day 10), the livers of C57BL/6J mice had more ^{14}C than livers of DBA/2J mice (the opposite was observed for fat tissue and muscle); however, the elimination half-time of the ^{14}C from this organ was 1.8 days in both strains. Elimination half-times 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 ^{14}C detected in tissues represented unmetabolized CDF. Four days following an oral dose of ^{14}C-labeled 2,3,4,7,8-pentaCDF, approximately 50% of the ^{14}C dose was in liver, 4–5% was in adipose, ~1% was in skin, and 1% was in skeletal muscle of C57BL/6N and 129/Sv mice (Diliberto et al. 1999). During the 4-day period following dosing, 26–33% of the dose was excreted; therefore, the liver accounted for approximately 60–80% of the body burden.

Hepatic uptake of pentaCDF in mice was shown to be dependent on expression of CYP1A2, which acts as an inducible binding protein for CDFs and CDDs (DeVito et al. 1997, 1998; Diliberto et al. 1995, 1997, 1999). Hepatic uptake of CDF congeners increases with CYP1A2 induction potency (DeVito et al. 1998; van Ede et al. 2013). Knockout of CYP1A2 decreased hepatic uptake by >10-fold and increased uptake in adipose by a factor of 5 (Diliberto et al. 1999). In mice expressing CYP1A2, observed liver/adipose concentration ratios ranged from 5 to 50, with the highest ratio observed for 2,3,4,6,8-pentaCDF, a relatively potent inducer of CYP1A2 (DeVito et al. 1998; Diliberto et al. 1999; van Ede et al. 2013). Retention of CDFs in liver in mice varies with chlorination. Liver elimination half-times of 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.

The distribution of CDFs in pregnant C57BW/6N mice and in the embryos was examined after oral administration of 800 µg ^{14}C-labeled 2,3,7,8-tetraCDF/kg in corn oil to the dams on GD 11 (Weber and Birnbaum 1985). Approximately 30 and 0.41% of the radioactivity per gram of tissue was found in the maternal liver and blood, respectively (only maternal liver and blood were analyzed), on GD 12; these percentages declined by half in both tissues on subsequent days (GD 14 and GD 13, respectively). The elimination half-time from the liver was estimated to be 1.5 days. Less than 0.01% of the radioactivity dose was detected in whole embryos at day 12, and no radioactivity could be detected at later times.

Distribution in Rats. Studies conducted in rats have also shown liver and adipose to be the major sites of uptake and retention of absorbed CDFs (Banks et al. 1990; Birnbaum et al. 1980; Brewster and Birnbaum 1987; Brewster et al. 1989; Golor et al. 1993; Körner et al. 2002; van Ede et al. 2014; Vanden Heuvel et al. 1994; Weber and Birnbaum 1985). After a single dose of 30.6 µg/kg of ^{14}C-labeled 2,3,7,8-tetraCDF to male Fischer-344 rats, the blood, liver, fat, skin, and muscle accounted for >90% of the retained ^{14}C dose at various times after dosing (Birnbaum et al. 1980). Nearly all of the ^{14}C detected in tissues was unchanged CDF. Loss of radioactivity from tissues could be described by exponential curves with one or more components. Half-times for the early components ranged from 0.02 days for blood and muscle to 0.45 days for skin. Late components had half-times ranging from 0.72 days for muscle to 11.1 days for

skin. Clearance from fat showed a single component with a half-time of 3.7 days. In other tissues, such as adrenals, kidneys, 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. Following oral dosing, liver/adipose concentration ratios of 2,3,4,7,8-pentaCDF ranged from 21 to 41 (Golor et al. 1993; Körner et al. 2002; van Ede et al. 2014). Retention of CDFs in liver in rats varies with chlorination. Liver retention of 2,3,4,7,8-pentaCDF was >50% of the dose, whereas retention of 2,3,7,8-tetraCDF ranged from 3 to 5% of the dose (Birnbaum et al. 1980; Brewster and Birnbaum 1987). Chlorine in substitution in position 4 appears to delay metabolic transformation (Burka et al. 1990). The liver retention half-time of 1,2,3,7,8-pentaCDF was 3.3 days, whereas the half-time for 2,3,4,7,8-pentaCDF was 108 days (van den Berg et al. 1989). 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.

Tissue distribution of CDFs was studied in male Fischer rats 3 days after receiving single applications of 31–340 µg/kg of ^{14}C-labeled 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, or 2,3,4,7,8-pentaCDF in acetone applied on a clipped area of the back (Brewster et al. 1989). For example, following the lowest dose, the liver had the most radiolabel per tissue (5.4% for 2,3,7,8-tetraCDF, 4.1% for 1,2,3,7,8-pentaCDF, 14.9% for 2,3,4,7,8-pentaCDF), 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 the dose in tissue decreased as dose increased, indicating decreased absorption at higher administered doses. The percentages of the administered 2,3,4,7,8-pentaCDF dose detected in the liver and adipose tissue were 72 and 6.7%, respectively, when expressed as a percentage of body burden. On a gram of tissue basis, the greatest concentration of radiolabel was detected in the liver. Of the three congeners evaluated, 2,3,4,7,8-pentaCDF had the highest concentration in the liver, followed by 2,3,7,8-tetraCDF and 1,2,3,7,8-pentaCDF. Consistent with 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.

Distribution in Guinea Pigs. After a single intravenous injection of ^{14}C-labeled 2,3,7,8-tetraCDF in guinea pigs (6 µg/kg) ^{14}C accumulated in liver, fat, muscle, and skin (Decad et al. 1981a). Analysis of these tissues suggested the presence of only parent compound. Three hours after dosing, a loss of ^{14}C from the liver could be accounted for by increases in adipose tissues 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. Experiments conducted in guinea pigs administered 6 µg

of labeled ^{14}C-labeled 2,3,7,8-tetraCDF/kg by gavage showed that most of the radioactivity (46%) accumulated in adipose tissue 3 days after dosing (Decad et al. 1981a). Liver, muscle, and skin accounted for about 16% each. After six or seven weekly oral doses of 2,3,7,8-tetraCDF at 1 μg/kg, the distribution of radiolabel in the tissues of guinea pigs was similar to that observed after a single oral dose (Decad et al. 1981a).

Mechanisms of Distribution. The mechanism by which CDFs cross biological membranes is not known. A contributing mechanism of distribution is partitioning into tissue lipids (Jackson et al. 1993; Maruyama et al. 2002). Accumulation of CDFs in liver has been shown to depend on expression of CYP1A2, which acts as an inducible binding protein for CDFs (DeVito et al. 1997, 1998; Diliberto et al. 1995, 1997, 1999).

3.1.3 Metabolism

Information on the metabolism of CDFs in humans can be derived from Yusho and Yu-Cheng patients since these subjects ingested contaminated rice oil in which ≈40 different CDF congeners were identified. 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. 1985a; Masuda et al. 1985).

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; Olson et al. 1994; van den Berg 1989). The major metabolic reactions include hydroxylation 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 P450 isoenzymes appear to catalyze the metabolic reactions (Olson et al. 1994; Tai et al. 1993; 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 et al. 1989). Female Sprague-Dawley rats were administered a single oral dose of several tetra- and pentaCDFs 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-tetraCDF) 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 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 et al. 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, indicating dehalogenation. 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.

Mechanisms of Metabolism. Studies conducted in microsomes from rat and human liver indicate that hydroxylation of 2,3,7,8-tetraCDF is mediated by the microsomal enzyme, CYP1A1. 2,3,7,8-tetraCDF was metabolized to 4-hydroxy-2,3,7,8-tetraCDF in human liver microsomes and recombinant yeast microsomes expressing CYP1A1, but not in yeast microsome expressing CYP1A2 (Tai et al. 1993). 2,3,7,8-TetraCDF was metabolized in liver slices, isolated hepatocytes, and liver microsomes from rats induced by pre-treatment with 2,3,7,8-tetraCDD (Olson et al. 1994; Tai et al. 1993). Hydroxylation of 2,3,7,8-tetraCDF in rat liver microsomes was inhibited by inhibitors or antibodies to CYP1A1, but not by inhibitors or antibodies to CYP1A2 (Tai et al. 1993).

3.1.4 Excretion

Excretion in Humans. Quantitative information on the routes of excretion of absorbed CDFs in humans was not available. Fecal excretion of CDFs can be measured in humans; however, these measurements represent the contributions of absorbed and unabsorbed CDFs (Jödicke et al. 1992; McLachlan 1993; Moser and McLachlan 2001; Schlummer et al. 1998; Schrey et al. 1998). In some studies, fecal excretion was found to exceed measured dietary intakes of CDFs (negative balance) suggesting that at least some of the fecal CDF was derived from body stores (Moser and McLachlan 2001; Schrey et al. 1998). Dietary intake-fecal excretion balance (net absorption) was found to correlate with concentrations of CDFs in blood lipid, suggesting a relationship between fecal excretion and body burden (Schlummer et al. 1998).

Numerous studies estimated rates of elimination of CDFs from longitudinal measurements of CDFs in serum or blood lipid (Table 3-2). Elimination rates reported in these studies do not necessarily distinguish elimination by metabolism or excretion of CDFs. These studies used a variety of approaches to estimate the half-times. Most studies estimated the half-times by fitting longitudinal data on observed blood CDFs to single exponential models, from which a half-time can be calculated as follows:

$$t_{1/2} = \frac{t \cdot \ln(2)}{\ln(\frac{C_0}{C_t})}$$

where t is time, C_0 is the concentration at time=0 and C_t is the concentration at time t. Several factors can affect these estimates, including the observation time (referred to as an epoch, or specific period of time) over which the half-times were estimated, ongoing exposures that occur during the epoch (e.g., baseline dietary exposures), age and body fat levels, measurement error, and statistical and/or kinetics models used in estimating half-times (Matsumoto et al. 2016; Milbrath et al. 2009). After cessation of a period of elevated exposure (e.g., occupational), blood CDF concentrations will decrease towards a value determined by current baseline exposure. As a result, measured half-times will depend on the epoch in which the blood concentrations are measured and its displacement from the period of elevated exposure (Matsumoto et al. 2009, 2016). Half times measured in epochs closer to the period of elevated exposure will reflect elimination of the body burden accumulated during the exposure, whereas half-times measured in epochs that are distant from the period of elevated exposure will be more greatly influenced by variations in baseline exposures (e.g., time trends in dietary intakes). For example, relatively long half-times ranging from 22 to 44 years were estimated for 2,3,4,7,8-pentaCDF based on blood measurements made 32 years after the Yusho incident. Half-times also appear to vary with the blood CDF concentration, which, in some studies, may have resulted

Table 3-2. Estimated Blood Elimination Half-Lives for Chlorodibenzofuran (CDF) Congeners in Humans

CDF congener	Subject[a]	Epoch[b] (years)	Interval[c] (years)	Half-life[d] (years)	Range	Reference
2,3,4,7,8-PentaCDF	Adult, occupational (n=43)	6	1	19.6[d]	NR	Flesch-Janys et al. 1996
1,2,3,4,7,8-HexaCDF				6.2[d]	NR	
1,2,3,6,7,8-HexaCDF				6.0[d]	NR	
2,3,4,6,7,8-HexaCDF				5.8[d]	NR	
1,2,3,4,6,7,8-HeptaCDF				3.0[d]	NR	
1,2,3,4,7,8,9-HeptaCDF				3.2[d]	NR	
2,3,4,7,8-PentaCDF	Adult, occupational (n=6)	6	16	13.9	4.6–23.1	Rohde et al. 1999
1,2,3,4,7,8-HexaCDF				8.7	4.1–17.3	
1,2,3,6,7,8-HexaCDF				5.8	3.6–9.2	
2,3,4,6,7,8-HexaCDF				9.9	8.7–12.6	
1,2,3,4,6,7,8-HeptaCDF				3.9	2.5–4.6	
2,3,4,7,8-PentaCDF	Adult, occupational (n=1)	6	2	7.2	NR	Schecter et al. 1990b
1,2,3,4,7,8-HexaCDF				4.4	NR	
1,2,3,6,7,8-HexaCDF				4.3	NR	
1,2,3,4,6,7,8-HeptaCDF				4.1	NR	
2,3,4,7,8-PentaCDF	Adult, Yusho (n=10)	8	22	9.6[e]	5.7–36	Ryan et al. 1993
1,2,3,4,7,8-HexaCDF				7.8[e]	4.3–54	
2,3,4,7,8-PentaCDF	Adult, Yusho (n=5)	15	14	7.7	5.2, 14.3	Masuda 2001
1,2,3,4,7,8-HexaCDF				5.1	3.9–6.9	
1,2,3,4,6,7,8-HeptaCDF				3.5	2.6–6.6	
2,3,4,7,8-PentaCDF	Adult, Yusho, >0.5 ppb (n=22)	5	32	21.7	NR	Matsumoto et al. 2009
2,3,4,7,8-PentaCDF	Adult, Yusho, 0.2–0.5 ppb (n=63)	5	32	44.0	NR	
2,3,4,7,8-PentaCDF	Adult, Yusho, 0.1–0.2 ppb (n=40)	5	32	25.6	NR	
2,3,4,7,8-PentaCDF	Adult, Yu-Cheng (n=3)	15	1	2.9	2.7–3.6	Masuda 2001
1,2,3,4,7,8-HexaCDF		15	1	3.5	2.7–3.6	
1,2,3,4,6,7,8-HeptaCDF		15	1	2.5	2.2–2.6	
2,3,4,7,8-PentaCDF	Adult, Yu-Cheng (n=3)	19	14	3.4	3.18–3.95	Ryan et al. 2001
1,2,3,4,7,8-HexaCDF		19	14	3.7	3.19– 4.01	

Table 3-2. Estimated Blood Elimination Half-Lives for Chlorodibenzofuran (CDF) Congeners in Humans

CDF congener	Subject[a]	Epoch[b] (years)	Interval[c] (years)	Half-life[d] (years)	Range	Reference
2,3,4,7,8-PentaCDF	Adult, Yu-Cheng (n=3)	9	1	2.2	1.9–2.3	Ryan et al. 1993
1,2,3,4,7,8-HexaCDF		9	1	2.6	2.1–2.9	
1,2,3,4,6,7,8-HeptaCDF		9	1	2.3	2.0–2.9	
2,3,4,7,8-PentaCDF		SS[g]	SS[g]	4.9	3.3–7.1[h]	Ogura 2004
1,2,3,4,7,8-HexaCDF	Adults, general (n=253)[f]	SS[g]	SS[g]	9.9	6.6–15[h]	
1,2,3,6,7,8-HexaCDF		SS[g]	SS[g]	17	11–26[h]	
1,2,3,4,6,7,8-HeptaCDF		SS[g]	SS[g]	4.8	3.2–7.2[h]	
2,3,4,7,8-PentaCDF	Infant (n=1)	1	0.1	0.30		Leung et al. 2006
2,3,4,7,8-PentaCDF		1	0.1	0.23		

[a]Studies are longitudinal in design, unless specified.
[b]The epoch is defined as the specific observation time for estimating half-times.
[c]Interval between end of high-level exposure and start of observations epoch.
[d]Mean, unless specified.
[e]Median.
[f]Cross-sectional study design.
[g]Estimated assuming steady-state blood concentration and estimated absorption rate from diet.
[h]95% confidence interval.

NR = not reported; SS = steady state

from effects of body burden on elimination or that the body burdens were approaching a new steady state governed by recent exposures (Leung et al. 2005; Matsumoto et al. 2009).

Some studies estimated the half-time based on cross-sectional blood CDF concentrations and estimates CDF intakes:

$$t_{1/2} = \frac{\ln(2)}{AF \cdot I}$$

where *AF* is an assumed absorption fraction for ingested CDF and *I* is the rate of intake of CDFs (Ogura 2004). Several factors can affect these estimates, including error in estimating long-term intake from short-term intake studies, subjects not being in steady state (which cannot be verified from cross-section observations), age and body fat levels, measurement error; and statistical models used in estimating parameters.

Table 3-2 includes several studies that estimated half-times for multiple congeners, of which the largest (n=43 adults) showed a trend for decreasing half-time with increasing chlorination (Flesch-Janys et al. 1996). This trend is also evident in several smaller studies (Masuda 2001; Rohde et al. 1999). In each of these studies, half-times were estimated from longitudinal measurements of blood CDF concentrations following cessation of a period of elevated exposure. The largest multiple-congener comparison (n=253) showed no consistent trend with chlorination (Ogura 2004). However, the Ogura (2004) study estimated half-times from cross-sectional data on blood CDF concentrations and dietary CDF intakes, rather than longitudinal measurements of blood CDF concentrations. This calculation assumes that the individuals were in a steady state and that the cross-sectional estimates of dietary intakes reflected long-term intakes of each individual, an assumption that is unlikely to be accurate for CDFs that have long half-times.

Excretion in Animals. Studies conducted in monkeys and rodents have shown that the primary routes of excretion of absorbed CDFs are feces and urine, with feces being the dominant route in monkeys, mice, and rats.

Excretion in Monkeys. In rhesus monkeys, during a 21-day period following a single intravenous dose of ^{14}C-labeled 2,3,7,8-tetraCDF/kg (30.7 µg/kg), 43% of the ^{14}C dose was excreted in feces and 8% was excreted in urine (Birnbaum et al. 1981). Fecal and urinary ^{14}C consisted of polar metabolites.

Excretion in Mice. Feces are the dominant route of excretion of CDFs in mice (Decad et al. 1981b; Diliberto et al. 1999). In C57BL/6J mice, during a 10-day period following a single intravenous dose of ^{14}C-labled 2,3,7,8-tetraCDF (30.6 µg/kg), 82% of the ^{14}C dose was excreted in feces and 13% was excreted in urine (Decad et al. 1981b). In DBA/2J mice, 56% of the dose was excreted in feces and 20% was excreted in urine (Decad et al. 1981b). Excreted ^{14}C in feces and urine consisted of parent compound and polar metabolites. In C57BL/6N and 129/Sv mice, during a 4-day period following a gavage dose of ^{14}C-labeled 2,3,7,8-tetraCDF (300 µg/kg) fecal excretion ranged from 21 to 30% of the of ^{14}C dose and urinary excretion ranged from 2 to 5% of the dose (Diliberto et al. 1999). Knockout of CYP1A2 expression in mice decreased hepatic retention of ^{14}C-labeled 2,3,7,8-tetraCDF and increased urinary excretion (25% of the ^{14}C dose; Diliberto et al. 1999). Pregnant C57BL/6N mice administered a single dose of 800 µg ^{14}C-labeled 2,3,7,8-tetraCDF/kg by gavage on GD 11 excreted 80% of the administered dose in the feces over a 3-day period; urinary excretion accounted for 5.4% of the dose (Weber and Birnbaum 1985).

Excretion in Rats. In rats, during a 5-day period following a single intravenous dose of ^{14}C-2,3,7,8-CDF (30.6 µg/kg) approximately 80% of the ^{14}C dose was excreted in feces and 5% in urine (Birnbaum et al. 1980). Bile was the major source of ^{14}C in feces. Fecal and urinary ^{14}C consisted of polar metabolites. In rats that received a gavage dose of ^{14}C-2,3,7,8-tetraCDF (31 or 306 µg/kg), approximately 70% of the ^{14}C dose was excreted in feces and approximately 1.5% was excreted in urine over a 3-day period (Birnbaum et al. 1980). Excretion of CDFs was studied in male Fischer-344 rats after receiving single dermal applications (3–340 µg/kg) of labeled ^{14}C-labeled 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, or 2,3,4,7,8-pentaCDF in acetone to a clipped area on their backs (Brewster et al. 1989). Elimination of ^{14}C occurred almost exclusively through the feces. For each congener, the relative amount of ^{14}C detected in the excreta decreased as the dose increased. At the lowest dose tested, fecal excretion accounted for 27% of the ^{14}C dose 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 ^{14}C from 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 µg 1,2,3,7,8-pentaCDF/kg. Approximately 90% of the 2,3,4,7,8-pentaCDF-derived fecal ^{14}C appeared to be parent compound. Excretion parameters for 2,3,4,7,8-pentaCDF-derived ^{14}C did not change as a function of age in male Fischer-344 rats (Banks et al. 1990). These results are consistent with the view that CDF congeners with chlorine substitution in position 4 (2,3,4,7,8-pentaCDF) are excreted slower than those unsubstituted in position 4 (2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF). A contributing factor to this difference may be slower metabolism of CDFs with chlorine substitution in position 4; 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.

Excretion in Guinea Pigs. Unlike monkeys, mice, and rats, fecal excretion was not the dominant excretory pathway for 2,3,7,8-tetraCDF in guinea pigs. In guinea pigs, during a 9-day period following a single intravenous dose of ^{14}C-labeled 2,3,7,8-tetraCDF (6 μg/kg), approximately 7% of the ^{14}C dose was excreted in feces and approximately 7% was excreted in urine. More than 90% of excreted ^{14}C was parent compound (Decad et al. 1981a). Over the same time period, following an oral dose of ^{14}C-labeled 2,3,7,8-tetraCDF (6 μg/kg), guinea pigs excreted 11% of ^{14}C dose in the feces and 3.3% in urine (Decad et al. 1981a). Slower elimination (metabolism and excretion) of 2,3,7,8-tetraCDF in the guinea pig compared to rats and mice, contributes to the higher toxic potency of 2,3,7,8-tetraCDF in guinea pigs (Decad et al. 1981a).

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

Much of the research on modeling the toxicokinetics of CDFs has focused on one-compartment models or statistical models for estimating elimination half-times (Campbell et al. 1996; Flesch-Janys et al. 1996; Kerger et al. 2007a, 2007b; Leung et al. 2007; Ogura et al. 2001; Portier et al. 1999; Rohde et al. 1999; Ryan et al. 2001; Schecter et al. 1990b; Tuomisto et al. 2016). Multicompartment models of varying complexity have also been developed simulating absorption, distribution, and elimination CDFs (Carrier et al. 1995a, 1995b; Maruyama et al. 2002, 2003).

Carrier et al. (1995a, 1995b) developed a three-compartment model simulating the absorption, distribution, and elimination of CDF congeners in humans and mammalian species. The model includes compartments representing blood, adipose, and liver tissues. The liver compartment simulates the

nonlinear relationship between the fraction of the body burden in liver (f_hC_b), which increases with increasing body burden (C_b; Carrier et al. 1995a). This is achieved by simulating binding of CDFs to an inducible protein in liver (e.g., CYP1A2) using parameters that can be adjusted to reproduce the observed dose relationships for the liver fraction of the body burden (f_hC_b), the adipose fraction of the body burden ($f_{at}C_b$), and the liver/adipose tissue concentration ratio (C_h/C_{at}) relationships. The model assumes instantaneous quasi-steady state for the absorbed dose, exchanges of free (unbound) CDF between all lipid fractions of compartments, and binding in the liver. Saturable binding in the liver is simulated as an instantaneous equilibrium:

$$C_x = \frac{C_x}{C_x \cdot K_D}$$

where C_x is the free (unbound) concentration and K_D is the binding dissociation constant. Induction of binding is assumed to have limited capacity with respect to the liver CDF concentration. This limitation is simulated with a Michaelis-Menten function of the liver fraction of the body burden:

$$f_h C_b = f_h^{min} + \frac{(f_h^{max} - f_h^{min}) \cdot C_b}{K + C_b}$$

where K is the induction constant and f_h^{min} and f_h^{max} are minimum and maximum (saturating) liver fractions, respectively. Elimination is assigned to the liver and governed by a first-order rate coefficient (day^{-1}). The model was evaluated with data on adipose concentrations of pentaCDF in Yu-Cheng patients (Ryan et al. 1993).

Maruyama et al. (2002, 2003) developed a six-compartment model for simulating absorption, distribution, and elimination of CDF congeners in humans. The model includes compartments representing blood, fat kidney, liver, muscle, skin, and a lumped compartment representing all other richly perfused tissues. Gastrointestinal absorption was governed by congener-specific absorption fractions that ranged from 87 to 99%. Exchanges of congeners between blood and tissues was assumed to be flow-limited; governed by tissue plasma flows (L/hour), the tissue-plasma partition coefficient, and the concentration gradient between blood and tissue venous blood. Elimination of congeners occurs via bile to feces, and kidney to urine; both are governed by first order-rate constants (day^{-1}), the concentration of congener in liver or kidney, and volume flow rates for bile or urine (L/day). The model does not simulate metabolism as a separate elimination pathway. The model was evaluated with data on blood and tissue congener levels in Yusho and Yu-Cheng patients (Iida et al. 1999a, 1999b).

3.1.6 Animal-to-Human Extrapolations

There are limited data on CDFs that allow for evaluating species differences. Potential differences have been more extensively investigated for CDDs; in particular, 2,3,7,8-TCDD. As discussed in ATSDR (2012), species differences in Ah receptor binding affinities have been reported between humans, rats, and mice. These data suggest that humans have approximately one-tenth the binding capacity compared to laboratory species. Comparisons of the EROD activity between humans and rats also suggest that 10-fold higher doses of 2,3,7,8-TCDD are needed to elicit the same response as observed in rats. Similarly, it appears that a higher 2,3,7,8-TCDD body burden is needed to induce increases in CYP1A1 gene expression in humans as compared to laboratory rodents. Although species differences have been found for 2,3,7,8-TCDD that suggest that humans may be less sensitive than rodents, it is not known whether these differences would also be found for CDF congeners. Comparisons of the endpoints of toxicity between those reported in the Yusho and Yu-Cheng cohorts and those reported in laboratory animals exposed to single CDF congeners or mixtures of congeners suggest a similarity in their toxicities. However, data are not available that would allow for dose-response comparisons.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health, nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high levels of CDFs are discussed in Section 5.7, Populations with Potentially High Exposures.

There are limited data on the toxicity of CDFs in children, and the toxicity is assumed to be similar to adults. As discussed in Section 2.16, *in utero* and/or lactational exposure to CDFs results in developmental effects. Adverse effects have been observed in the children of women exposed to CDFs in the Yusho and Yu-Cheng incidents. These effects included skin, nail, gingival hyperpigmentation, 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 are similar to those observed in adults. Other reported effects in these children include decreases in birth weight (Funatsu et al. 1971; Lan et al. 1987; Rogan 1989; Taki et al. 1969; Yamaguchi et al. 1971), decreased muscular development (Guo et al. 1994a), cognitive delays (Chen et al. 1992; Guo et al. 1995), and possibly reproductive effects (Guo et al. 2000; Hsu et al. 2005; Yang et al. 2005). Studies in laboratory animals report several developmental effects including hydronephrosis, cleft palate, fetal mortality, decreases in fetal weight, decreases in thymus weight, and impaired development of the reproductive system (Birnbaum et al. 1987a, 1987b; Couture et al. 1989; Madsen and Larsen 1989; Salisbury and Marcinkiewicz 2002; Taura et al. 2014; Weber et al. 1984, 1985).

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers 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, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to CDFs are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for CDFs from this report are discussed in Section 5.6, General Population Exposure.

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 effect caused by CDFs are discussed in Section 3.3.2.

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 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

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 tissues, and are present in whole blood, serum, plasma, and human milk. High amounts of CDFs are also found in the liver. In general, CDFs have been found at lower concentrations in all other tissues examined to date. Serum and adipose tissue CDF levels are indicators of exposure that can provide an estimate of body burden because, as discussed in Section 3.1.2, 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 weight basis are higher in liver than in adipose tissue (Beck et al. 1990a; 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 varies 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 victims (Ryan et al. 1993). Sampling was conducted over a 8-year period starting 2 years after the incident. The same investigators (Ryan et al. 1993) calculated a median elimination half-time of 10 years for the same

congeners in Yusho victims. In this case, sampling was conducted over an 8-year period, but starting 14 years after the poisoning had occurred.

Hair analysis can be a useful method for identifying recent exposure to CDFs in ambient air (Schramm et al. 1992). Hair levels appear to reflect body burden and atmospheric burden (Nakao et al. 2002; Tirler et al. 2001). When the concentrations in blood were compared to hair levels in a study of six adults, a correlation between the two was only found for 2,3,4,7,8-pentaCDF; no correlations were found for the other congeners (Nakao et al. 2002). Another study found that the congener profile in hair differed from that of blood or breast milk levels (Tirler et al. 2001). For example, the ratio of 1,2,3,7,8-pentaCDF to 2,3,4,7,8-pentaCDF in blood is at least 1:10, but in hair the ratio was approximately 1:2. The congener pattern in hair was similar to skin lipid, environmental samples, and spruce needles.

3.3.2 Biomarkers of Effect

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 can also be induced by other chloroaromatic compounds (e.g., CDDs) that act by a common Ah receptor-mediated mechanism (see Section 2.20). As discussed in Section 3.3.2, 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).

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.

3.4 INTERACTIONS WITH OTHER CHEMICALS

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, 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 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 (DeVito et al. 1993; Pluess et al. 1988a) with varying results depending upon the endpoint assessed (Eadon et al. 1986; Nagao et al. 1993; Pluess et al. 1988a).

Additive effects, as well as the 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. 1988a). 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.

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 GDs 9–11 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 on 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. 1987b). 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 µmol 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 1,000/1. 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. The investigators suggested that the 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 SRBCs (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 25–350% 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.