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

3.1 TOXICOKINETICS

Data regarding toxicokinetics of CDDs in humans are limited to information derived from exposures that occurred after industrial accidents, exposures of Vietnam veterans, and ingestion of large doses of 2,3,7,8-TCDD.

- Humans can absorb CDDs by the inhalation, oral, and dermal routes of exposure. CDDs, when administered orally, are well absorbed by experimental animals, but they are absorbed less efficiently when administered by the dermal route. Limited data in rats showed that transpulmonary absorption of 2,3,7,8-TCDD may be at least as efficient as oral absorption. In a volunteer, >86% of the administered single oral dose appeared to have been absorbed. In general, absorption is vehicle-dependent and congener-specific. Passage across the intestinal wall is predominantly limited by molecular size and solubility. These parameters are most significant for hepta- and octachlorinated congeners, which exhibit decreased absorption in mammals.

- The predominant CDD carriers in human plasma are serum lipids and lipoproteins, but chlorine substitution plays a role in the distribution in these fractions. For most mammalian species, the liver and adipose tissue are the major storage sites of CDDs; in some species, skin and adrenals also can act as primary deposition sites. 2,3,7,8-Substituted CDDs are the predominant congeners retained in tissues and body fluids. Tissue deposition is congener-specific and depends on the dose, the route of administration, and age.

- CDDs are very slowly metabolized by the microsomal monooxygenase system to polar metabolites that can undergo conjugation with glucuronic acid and glutathione.

- The major routes of excretion of CDDs are the bile and the feces; smaller amounts are excreted via the urine. In mammalian species, lactation is an effective way of eliminating CDDs from the liver and other extrahepatic tissues.

- Physiologically based pharmacokinetic (PBPK) models have been developed to describe disposition of 2,3,7,8-TCDD in humans and animals. Some of these models included parameters to describe complex interactions of 2,3,7,8-TCDD with cellular proteins that lead to specific biological responses.

3.1.1 Absorption

Inhalation Exposure. No quantitative data were located regarding absorption of CDDs in humans following inhalation exposure. Data on levels of CDDs in blood from populations with above-background exposures (occupational, accidental) suggest that transpulmonary absorption occurs in humans.

Systemic effects (hepatic aryl hydrocarbon hydroxylase [AHH] and CYP induction, hepatic histological alterations) were observed in rats following a single intratracheal instillation of 2,3,7,8-TCDD in a corn oil vehicle or as a laboratory-prepared contaminant of gallium oxide particles (Nessel et al. 1990). In a subsequent study, the same group of investigators (Nessel et al. 1992), using a similar protocol, found that the relative pulmonary bioavailability of 2,3,7,8-TCDD on respirable soil particles was 100% as compared to the gallium oxide vehicle. At 1- and 7-days post-treatment, 13.9 and 11.9% of the administered dose were detected in the liver, respectively, and this was similar to the percentage found after instillation of contaminated gallium oxide particles. Twenty-eight days after treatment, 5.2% of the administered dose was detected in the liver from soil-treated rats and 2.9% of the administered dose was detected in the liver from gallium oxide-treated rats, suggesting that redistribution and retention of 2,3,7,8-TCDD differed in the two treatment groups. Diliberto et al. (1996) reported that 3 days after intratracheal application of a single dose of 0.32 μg 2,3,7,8-TCDD/kg to male Fischer-344 rats, 95% of the applied dose was absorbed, suggesting that inhalation can be an effective route of exposure. The extent of inhalation absorption was higher than when the same dose was administered orally (88%) or dermally (40%). The available data suggest that inhaled CDDs will be absorbed. However, the degree of absorption and the rate will depend on the media on which the CDDs are adsorbed and the degree of chlorination.

Oral Exposure. The absorption of 2,3,7,8-TCDD was estimated to be >87% in a volunteer following ingestion of a single radioactively labeled dose of 0.00114 μg 2,3,7,8-TCDD/kg in corn oil (Poiger and Schlatter 1986). Absorption of several CDDs (2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD; 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,9-HpCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD) from food was examined in seven volunteers using a mass balance protocol, collecting food (normal diet; not controlled) and feces over a 3-day period (Schlummer et al. 1998). Volunteers did not have any history of occupational or accidental exposure to CDDs. The highest net absorption observed for an individual volunteer was 62% for 2,3,7,8-TCDD in a 28-year-old male. However, estimates of absorption were highly variable and, in some individuals, net excretion rather than net absorption was observed. The study authors suggested that variability was related, in part, to the variability of food content of CDDs. Using a similar study design, the absorption of several CDDs (same as those evaluated by Schlummer et al. 1998) was estimated in five volunteers for both low- and high-CDD intake diets (Moser and McLachlan 2001). For high-intake diets, the net absorption of 2,3,7,8-TCDD, PeCDD, and HxCDD was >80%, with lower net absorption for HpCDDs (approximately 70%) and OCDD (approximately 50%). For low-intake diets, the net absorption of most CDDs could not be detected; findings are consistent with

net excretion of CDDs from body stores under low-intake conditions. Data regarding absorption of CDDs from human milk in nursing infants are provided in Section 3.1.4.

Gastrointestinal absorption of radiolabeled 2,3,7,8-TCDD has been investigated in rodents. About 73.5% of the total dose of 2,3,7,8-TCDD (administered by gavage in corn oil vehicle) was absorbed in Syrian hamsters, the species most resistant to acute 2,3,7,8-TCDD toxicity (Olson et al. 1980b). In Sprague-Dawley rats given a single gavage dose of 50 μg/kg 2,3,7,8-TCDD in corn oil, at least 70% was absorbed (Piper et al. 1973). Rose et al. (1976) found a mean of 84% of a single gavage dose of 1 μg/kg absorbed within a day in a similar study and a steady-state body burden was achieved after dosing with 0.01, 0.1, or 1 μg/kg in corn oil, 5 days/week for 7 weeks. When [^{14}C]-2,3,7,8-TCDD was fed to Sprague-Dawley rats at 0.35 or 1 μg/kg/day in the diet for 42 days, about 60% of the consumed dose was absorbed (Fries and Marrow 1975). Intestinal absorption of 2,3,7,8-TCDD did not vary with age of Fischer-344 rats (13 weeks, 13 or 26 months) when *in vivo* absorption was studied with an *in situ* intestinal perfusion technique (Hebert and Birnbaum 1987). When ICR/Ha Swiss mice were given a single dose of radioactively labeled 2,3,7,8-TCDD, 67–76% of the administered dose was excreted in feces and 1–2% was excreted in urine within the first 24 hours (Koshakji et al. 1984). The study authors concluded that most of the dose was not absorbed.

Gastrointestinal absorption of 2,3,7,8-TCDD may differ depending on the vehicle used. When hepatic concentrations were used as a measure of absorbed dose, the levels observed in rats 24 hours after 2,3,7,8-TCDD administration in 50% ethanol were higher than in an aqueous suspension of soil (Poiger and Schlatter 1980). Use of activated carbon as a vehicle almost completely eliminated 2,3,7,8-TCDD absorption. It was further demonstrated that the absorption of 2,3,7,8-TCDD from the gastrointestinal tract of rats was ≈50% less from contaminated soil than from corn oil (Lucier et al. 1986), which is supported by the finding that 2,3,7,8-TCDD-contaminated soil was less toxic to guinea pigs than an equivalent amount of 2,3,7,8-TCDD in oil (Umbreit et al. 1985). The more highly chlorinated CDD congeners are absorbed from the gastrointestinal tract to a lesser extent than 2,3,7,8-TCDD. Gastrointestinal absorption of OCDD was <10% of the administered dose in Sprague-Dawley and Fischer-344 rats following single or repeated (3-week) exposures by gavage in an oil vehicle (Birnbaum and Couture 1988; Norback et al. 1975). Low doses (50 μg/kg) in a *o*-dichlorobenzene:corn oil (1:1) vehicle were found to give the best oral bioavailability for this extremely insoluble compound (Birnbaum and Couture 1988). The bioavailability of CDDs (2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD, and 1,2,3,7,8,9-HxCDD) to rats was lower on fly ash (0.4% for 2,3,7,8-TCDD) as compared to extracts of the same fly ash administered in an oily vehicle (45% for 2,3,7,8-TCDD) (Van den Berg et al. 1983,

1987a). The differences in hepatic levels between fly ash- and extract-treated rats were greater for the more highly chlorinated congeners.

Additional studies have evaluated the oral bioavailability of CDDs in soil relative to bioavailability in a reference material (relative bioavailability or RBA), such as corn oil, has been evaluated using several animal models, including rats (Budinsky et al. 2008; Finley et al. 2009; Lucier et al. 1986; Shu et al. 1988), guinea pigs (McConnell et al. 1984; Umbreit et al. 1986a; Wendling et al. 1989), rabbits (Bonaccorsi et al. 1984), and swine (Budinsky et al. 2008; Wittsiepe et al. 2007). Results of all studies show that the RBA of CDDs in soil is <100%, indicating that bioavailability of CDDs in soil is reduced compared to bioavailability of CDDs in the reference material. Relative bioavailability values for CDDs in soil were highly variable, ranging from <1 to 66% (Budinsky et al. 2008; Umbreit et al. 1986a). Variability in RBA values may be related to several factors, including differences in soil characteristics, CDD congener composition of soil, experimental protocol, and/or species differences.

Dermal Exposure. No quantitative data were located regarding absorption of CDDs in humans following dermal exposure. However, based on data from studies with structurally related chemicals, it is reasonable to assume that CDDs are absorbed by this route. Furthermore, data on levels of CDDs in blood from populations with above-background exposures (i.e., occupational, accidental) also suggest that dermal absorption occurs in humans. Due to the relatively low vapor pressure and high lipid solubility, dermal uptake of 2,3,7,8-TCDD in the workplace may be a significant source of occupational exposure (Kerger et al. 1995).

Kerger et al. (1995) examined the potential contribution of dermal exposure to 2,3,7,8-TCDD for three different occupational exposure scenarios: (1) trichlorophenoxy herbicide manufacturing worker (20-year exposure); (2) contract maintenance mechanic exposed by repairing a trichlorophenol reactor after an explosion accident (6-week exposure); and (3) trichlorophenoxy applicator handling only diluted trichlorophenoxy herbicides (seasonal exposure for 20 years). In their evaluation, the study authors used a conceptual model of workplace exposure, dermal bioavailability/uptake calculations, and simple pharmacokinetic modeling techniques (details of the model were not provided). The contribution of background uptake of 2,3,7,8-TCDD from dietary sources in the United States was accounted for in the estimates of steady-state adipose concentrations. The results of the modeling showed that considerable occupational uptake can occur following both long-term continuous exposure and short-term high exposure. In the former case, occupational uptake can be distinguished from background exposures when

body burden is measured within a 10-year period following cessation of exposure. In contrast, seasonal exposure to dilute 2,3,7,8-TCDD residues may result in little or no change in 2,3,7,8-TCDD body burden.

The *in vitro* penetration of [^{3}H]-labeled 2,3,7,8-TCDD into human cadaver skin was studied at concentrations of 6.5 and 65 ng 2,3,7,8-TCDD/cm^2 of skin (Weber et al. 1991a). Two vehicles were used: (1) acetone to simulate exposure to 2,3,7,8-TCDD as a dry material and (2) mineral oil to simulate exposure in an oily medium. The experiments were conducted in intact skin and in skin with stripped stratum corneum, and penetration was monitored for 30, 100, 300, and 1,000 minutes. The results showed that acetone as a vehicle allowed 2,3,7,8-TCDD to penetrate deeply into the loose surface of the lamellae of the stratum corneum, but there was little further penetration. On the other hand, mineral oil appeared to compete with lipophilic constituents of the stratum corneum for 2,3,7,8-TCDD, thus slowing its penetration even more. Removal of the stratum corneum increased the amount of 2,3,7,8-TCDD absorbed into layers of the skin. Rates of absorption were calculated in two ways: (1) a worst-case scenario where 2,3,7,8-TCDD absorbed into any layer of skin, including the stratum corneum, was used for analysis and (2) a physiological approach where only the amount of 2,3,7,8-TCDD that had penetrated beyond the epidermis into the region of dermal vascularization was considered absorbed. In the former case, the stratum corneum appeared to mediate dermal absorption of 2,3,7,8-TCDD since the rates decreased when stripped skin was exposed to 2,3,7,8-TCDD. With the physiological approach, the rate of absorption was a function of the amount applied, suggesting that the rate of absorption per unit time was a first-order function. The amount of 2,3,7,8-TCDD that penetrated the skin also correlated with exposure duration. The rates of 2,3,7,8-TCDD penetration with acetone as vehicle were 100–800 pg 2,3,7,8-TCDD per hour-cm^2 (worst-case scenario), or 6–170 pg per hour-cm^2 with the physiological approach. The corresponding values with mineral oil as a vehicle were 20–220 and 1.4–18 pg per hour-cm^2, respectively.

Data regarding dermal absorption of CDDs in animals are limited. Dermal absorption of 2,3,7,8-TCDD (70 mg total dose in acetone or in a low organic soil) was evaluated following application to shaved skin of female Sprague-Dawley under occluded conditions (Roy et al. 2008). After 96 hours, dermal absorption of 2,3,7,8-TCDD was 77.6 and 16.3% for acetone and soil applications, respectively. When 200 pmol 2,3,7,8-TCDD was applied to the skin of Fischer-344 rats, absorption followed first-order kinetics with an absorption rate constant of 0.005 hour^{-1} (Banks and Birnbaum 1991). Within 120 hours postexposure, about 0.026 µg 2,3,7,8-TCDD was absorbed (<50% of the applied dose); at each interval of measurement, about 70% of detected radioactivity on the skin could be removed by swabbing with acetone. About 15% of the dose was detected in the liver of rats 24 hours after dermal exposure to 26 ng of 2,3,7,8-TCDD in 50% methanol (Poiger and Schlatter 1980). It was estimated that the amount

absorbed from the dermal exposure represents ≈40% of the amount absorbed from an equivalent oral dose. Absorption of 2,3,7,8-TCDD was significantly reduced by application in Vaseline or polyethylene glycol and practically eliminated in soil or activated carbon. Dermal absorption of radioactively labeled 2,3,7,8-TCDD in soil vehicle was reported to be only 1% of the administered dose during a 24-hour contact in rats (Shu et al. 1988). The dermal absorption of 2,3,7,8-TCDD after 4 hours of contact was about 60% of that after 24-hour contact. The uptake was not influenced by the 2,3,7,8-TCDD concentration in soil, nor were there any differences between normal and hairless rats.

Dermal absorption in rats was found to be age-related. Banks et al. (1990) found that in Fischer-344 rats, percutaneous absorption was decreased in middle-aged (36-week-old) and senescent (120-week-old) rats compared to that in young adults (10-week-old) 72 hours after application of a dose of 40 nmol (approximately 12.9 μg) of [^{3}H]-labeled 2,3,7,8-TCDD. The study authors suggested a decrease in blood flow through the skin between 3 and 4 months of age as a possible explanation for their findings. In a subsequent and similar study, the same group of investigators examined the dermal absorption of 2,3,7,8-TCDD in 3-, 5-, 8-, 10-, and 36-week-old Fischer-344 rats 72 hours after application of 200 pmol 2,3,7,8-TCDD in acetone (Anderson et al. 1993). Dermal absorption was greatest in 3-week-old rats (approximately 64% of the applied dose) and decreased to about 40% of the applied dose in 5-, 8-, and 10-week-old rats and to about 22% in 36-week-old rats. In each age group, 70–80% of the radioactivity remaining at the application site 72 hours after dosing could be removed with acetone swabs.

3.1.2 Distribution

As discussed in Section 2.1, occupational or environmental human exposure to CDDs is not readily classifiable as to route of exposure. However, it has been estimated that food contributes over 90% of background exposure to CDDs. Human data regarding distribution obtained at autopsy indicated that accumulation in the liver following low levels of exposure is based, in part, on lipid solubility (Leung et al. 1990a; Watanabe et al. 2013). However, this may not be the case with higher exposure levels that cause hepatic enzyme induction. When human hepatic and adipose tissues were examined for the presence of 2,3,7,8-TCDD, the concentration detected in the liver was about 1/10 of that in the adipose tissue on a whole-tissue-weight basis. However, on the basis of the total tissue lipid, the concentration in adipose tissue lipid was one-half that in the liver lipid (Thoma et al. 1990). Watanabe et al. (2013) measured TEQs (CDDs, CDFs, PCBs) in human adipose and liver autopsy samples. TEQ concentrations (per g lipid) in adipose and liver samples were similar and were 1.3 and 1.5 times higher in males compared to females. In this same study, liver/adipose concentration ratios for OCDD and

1,2,3,6,7,8-HxCDF increased with increasing levels of hepatic CYP1A2. It was further demonstrated that over a wide range of concentrations, the serum 2,3,7,8-TCDD levels highly correlated with adipose tissue 2,3,7,8-TCDD levels when both were expressed on a lipid weight basis (Patterson et al. 1988). Adipose tissue serves as a storage depot for 2,3,7,8-TCDD in the body, and detectable levels (up to 20.2 ppt) were found in the general population with no known risk of high exposure to CDDs (Andrews et al. 1989). Studies conducted in mice have shown that 2,3,7,8-TCDD stored in adipose tissue grafts can be released and distributed to other tissues (Joffin et al. 2018). An average concentration of 2,3,7,8-TCDD in serum lipid of 5.38 pg/g has been estimated for the U.S. population (Orban et al. 1994). The distribution of highly chlorinated CDDs among tissue lipid fractions is not equal. For example, the distribution of OCDD is 12:1 (Thoma et al. 1990) between liver and adipose tissue lipid factions and 2:1 between serum and adipose tissue lipid fractions (Schecter et al. 1990b). A study conducted in Norway found that men and women who had similar dietary congener profiles had different serum congener profiles (Knutsen et al. 2011). In this study, the results of a regression analysis of factors influencing congener profiles suggested that being female was associated with lower levels of 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, and 1,2,3,4,7,8,9-HpCDF.

Increased adipose tissue levels of CDDs were reported in populations with known high residential or occupational exposure (Beck et al. 1989b; Fingerhut et al. 1989; Patterson et al. 1989b; Schecter et al. 1994c). For example, high levels of 2,3,7,8-TCDD were found in fat (42–750 ppt) and serum lipid (61–1,090 ppt) of Missouri chemical workers (Patterson et al. 1989b). Measurable CDDs and CDFs levels were reported in the liver tissue of human stillborn neonates, suggesting that the transplacental intrauterine transfer of these persistent chemicals resulted from environmentally exposed mothers (Schecter et al. 1990b). In addition, CDDs are distributed to human milk (i.e., Fürst et al. 1994; Schecter et al. 1987a, 1987b, 1989e) and numerous studies have published concentrations of various congeners in human milk samples (see Section 5.6). Levels of CDDs in human milk have been found to be significantly and positively associated with proximity of residence to waste sites and to dietary fat intake per week (Schlaud et al. 1995).

Inhalation Exposure. The tissue distribution of 2,3,7,8-TCDD-derived radioactivity was examined in male Fischer-344 rats 3 days after intratracheal application of a single dose of 0.32 µg 2,3,7,8-TCDD/kg (Diliberto et al. 1996). The liver and adipose tissue were the major tissue depots for 2,3,7,8-TCDD-derived radioactivity, with 32.9 and 14.9% of the applied dose distributing to these respective tissues. The skin (ear) and muscle followed with 4.3 and 1.3%, respectively. All other tissues had <0.5% of the

administered dose. The 2/1 liver/adipose ratio was in contrast to the approximately 1/1 ratio found after gavage administration of the same dose.

Oral Exposure. Following an ingested dose of [^{3}H]-2,3,7,8-TCDD of 0.00114 µg/kg by a volunteer, the concentrations of 2,3,7,8-TCDD in the adipose tissue were 3.09 and 2.86 pg/g at 13 and 69 days following exposure, respectively (Poiger and Schlatter 1986). The study authors estimated that about 90% of the body burden was distributed to the fatty tissue. Increased radioactivity was detected in the blood only during the first 2 days postexposure; no radioactivity was detected in serum lipid after 5 days, but was in the feces for several months.

Studies in animals have shown that 2,3,7,8-TCDD distributes preferentially to the liver and adipose tissue. Following single gavage administration of 50 or 100 ng/kg of 2,3,7,8-TCDD in corn oil to female Harlan Sprague-Dawley rats, TCDD tissue concentrations in blood, lung, liver, and adipose were measured at several time intervals up to 150 days (NTP 2006). The highest peak tissue concentration (per gram of tissue) was observed in liver, followed by adipose > lung ≈ blood. Peak blood levels of 2,3,7,8-TCDD were observed within 24 hours of dosing, decreasing to nondetectable levels after 15 days; results were consistent with rapid distribution to tissues. Peak liver concentration was observed within 24 hours of dosing, whereas peak adipose concentration was observed in 20–40 days. In Sprague-Dawley rats, the highest levels of radioactivity (expressed as percentage of dose per gram of tissue) were located in the liver (3.18, 4.49, and 1.33% at days 3, 7, and 21 post-exposure, respectively) and adipose tissues (2.6, 3.22, and 0.43% at days 3, 7, and 21, respectively) following a single oral dose of labeled 2,3,7,8-TCDD at 50 µg/kg (Piper et al. 1973). Much smaller amounts were found in muscles, testes, lungs, stomach, and other organs. In male Fischer-344 rats administered a single gavage dose of 0.32 µg 2,3,7,8-TCDD/kg, 24.4 and 26.2% of the administered dose was found in the liver and adipose tissue, respectively, 3 days after dosing (Diliberto et al. 1996); skin and muscle had 7.3 and 1.8%, respectively. 2,3,7,8-TCDD accumulated mainly in the liver and adipose tissue, with smaller amounts in the brain of pregnant Wistar rats after 10 daily doses of 2 µg/kg (Khera and Ruddick 1973). Similarly, the highest levels of radioactivity were found in the liver, adipose tissue, and adrenals of Golden Syrian hamsters after a single gavage dose of 650 µg/kg labeled 2,3,7,8-TCDD (Olson et al. 1980b). In addition, about 36% of the total radioactivity administered remained in the adipose tissue by day 45 postexposure in Hartley guinea pigs; only about 7% (each) was found in the liver, pelt, and skeletal muscles and carcass (Olson 1986). When pregnant NMRI mice were exposed to a single oral, intraperitoneal, or subcutaneous dose of 2,3,7,8-TCDD, hepatic levels were about the same, indicating that there is no major first-pass effect after oral 2,3,7,8-TCDD exposure (Nau and Bass 1981). Liver, then adipose tissue and skin, were

the major depots of OCDD in Fischer-344 rats treated with single oral doses of this congener (Birnbaum and Couture 1988). One day following a single oral dose of [^{3}H]-labeled 2,3,7,8-TCDD (12.5 ng/kg) administered to female Long-Evans rats, the largest percentages of the administered radioactivity dose were found in liver (46%) adrenal gland (20%), adipose (24%), lung (14%) and thymus (10%) (Yonemoto et al. 2005). In this same study, the distribution of radioactivity following a single dose of [^{3}H]-labeled 2,3,7,8-TCDD administered to pregnant rats late in gestation was similar to that of non-pregnant rats, with the largest percentages of dose found in liver (11.8%) and adipose tissue (3.65%). A similar distribution of radiolabelled 2,3,7,8-TCDD was found in pregnant and adult male rats following a single dose of [^{14}C]-2,3,7,8-TCDD (10 μg/kg), with the largest percentage of the dose found in liver (Ishida et al. 2010).

The dose- and time-dependent tissue distribution of 2,3,7,8-TCDD in mice has been examined (Diliberto et al. 1995, 1998, 1999, 2000; Hakk et al. 2009; van Birgelen et al. 1996). Results show that distribution to liver and adipose tissue is dose-dependent; at lower doses, distribution (as a percentage of the administered dose) to adipose tissue is greater than to liver, whereas at higher doses, distribution to liver is greater than to adipose tissue. A typical example of the patterns for dose- and time-dependent distribution of 2,3,7,8-TCDD is provided in a study by Diliberto et al. (1995). In this study, female B6C3F1 mice were administered a single dose of 0.1, 1, or 10 μg [^{3}H]-2,3,7,8-TCDD/kg by gavage in corn oil and the distribution of radioactivity was followed in 18 tissues for up to 35 days after dosing. The results showed dose-dependent distribution of 2,3,7,8-TCDD-derived radioactivity in all tissues. The highest concentrations of radioactivity were found in liver and adipose tissues, and both tissues accounted for 50% of the body burden. Relatively high concentrations of 2,3,7,8-TCDD-derived radioactivity were also found in skin, adrenal glands, thyroid, pancreas, olfactory epithelium, spleen, mesenteric lymph nodes, thymus, lung, and bone marrow. The liver concentration of radioactivity increased disproportionally with increasing doses, whereas relative concentration and percentage dose/total tissue in extrahepatic tissues decreased with increasing dose and over time. Liver/adipose tissue concentration ratios were shown to be dose- and time-dependent. At the low, mid-, and high dose, the ratios were 0.6–0.2, 2.3–0.5, and 3.1–1.4 over time, respectively. This variation over time was thought to have been due to redistribution of 2,3,7,8-TCDD between the two storage sites and/or hepatic metabolism and subsequent excretion. Dose-dependence of distribution of 2,3,7,8-TCDD and TEQ to liver and adipose tissue appears to be related to induction of CYP1A2, a protein that is under AhR transcriptional regulation and binds to 2,3,7,8-TCDD (Diliberto et al. 1998, 1999, 2000; Hakk et al. 2009; Watanabe et al. 2010).

The effect of age of the animal on 2,3,7,8-TCDD tissue distribution has also been examined. Pegram et al. (1995) administered a single dose of 0.015, 0.5, or 15 µg [^{3}H]-2,3,7,8-TCDD/kg to 10-week-old and 28-month-old male C57BL/6N mice and monitored 2,3,7,8-TCDD-derived radioactivity in blood, liver, skin, kidney, and muscle 7 days after dosing. The results showed that in young mice given the low and high dose, the concentration of 2,3,7,8-TCDD in blood relative to all other tissues was significantly greater than in older mice. Also, in older mice, the concentration of 2,3,7,8-TCDD in skin and the percentage of the dose in the skin were greater than in the young mice. The same trend was observed in kidney and muscle. The concentration of 2,3,7,8-TCDD in liver, as well as the percentage of the dose in the liver, were greater in younger animals as compared to older animals at both the mid- and high doses. In both younger and older mice, the ratios of liver to adipose tissue increased with increasing doses. According to the study authors, the higher hepatic concentration of 2,3,7,8-TCDD in younger mice could be due to the older mice having a larger fat compartment, such that the hepatic 2,3,7,8-TCDD sequestering action of CYP1A2 or other inducible binding factors may have been less effective in the more obese older mice. In addition, decreased perfusion in the liver and adipose compartments in the older mice may have limited the effectiveness of hepatic 2,3,7,8-TCDD accumulation. The greater accumulation of 2,3,7,8-TCDD in the skin, muscle, and kidney from older mice was attributed to altered perfusion and possibly greater lipid infiltration in these tissues.

The subcellular distribution of 2,3,7,8-TCDD-derived radioactivity in the liver, lungs, and kidneys from female Sprague-Dawley rats and B6C3F1 mice was studied by Santostefano et al. (1996). In the liver of rats given a single oral dose of 0.1, 1, or 10 µg [^{3}H]-2,3,7,8-TCDD/kg, radioactivity accumulated equally in the supernatant (S9, cytosol, and microsomes) and pellet (P9, nucleus, lysosomes, and mitochondria) fractions; within the S9 fraction, accumulation was predominantly in the microsomal fraction. In contrast, in kidneys and lungs, radioactivity accumulated preferentially in P9, but radioactivity detected in S9 was mostly in the cytosolic fraction. The pattern of distribution of radioactivity in liver and lungs from mice was similar to that found in rats, but in mice kidneys, 2,3,7,8-TCDD detected in S9 was equally distributed between the microsomal and cytosolic fractions. Accumulation of 2,3,7,8-TCDD in the various fractions in this single-dose study was not dose-dependent. The investigators also conducted a 17-week oral dosing study in B6C3F1 mice given 1.5 or 150 ng/kg that showed that increasing the dose resulted in equal accumulation between liver S9 and P9 fractions, whereas the kidney P9 had the most radioactivity regardless of the dose. In addition, liver S9 accumulated 2,3,7,8-TCDD in the microsomal fraction, whereas kidney S9 accumulated predominantly in the cytosol. These results are consistent with the hypothesis that hepatic microsomal sequestration of 2,3,7,8-TCDD is mediated by CYP1A2, a dioxin-inducible protein. This hypothesis was subsequently confirmed by experiments in transgenic mice

lacking expression of Cyp1a2 (Cyp1a2$^{-/-}$) (Diliberto et al. 1997). These mice, as judged by 2,3,7,8-TCDD liver/fat concentration ratios, failed to sequester 2,3,7,8-TCDD in the liver after administration of a single dose of 2,3,7,8-TCDD.

The distribution of CDDs under steady-state or near steady-state conditions has been studied in intermediate-duration oral exposure studies (Birnbaum and Couture 1988; Birnbaum et al. 1989a; DeVito et al. 1998; Diliberto et al. 2001; Fries and Marrow 1975; Laurent et al. 2005; Norback et al. 1975). In female B6C3F1 mice administered via gavage [^{3}H]-2,3,7,8-TCDD (1.5 or 150 ng/kg/day) in corn oil 5 days/week for 13 weeks, radioactivity was detected in all tissues examined (blood, adipose tissue, liver, kidneys, lungs, skin, muscle, spleen, and thymus), with the highest tissue concentrations in liver and adipose tissue (Diliberto et al. 2001). As demonstrated in single-dose studies (discussed above), at the lower dose, distribution (as a percentage of the administered dose) to adipose tissue was greater than to liver, whereas at the higher dose, distribution to liver was greater than to adipose tissue. In female B6C3F1 mice administered 2,3,7,8-TCDD (1.5–150 ng/kg/day) or 1,2,3,7,8-PeCDD (90–9,000 ng/kg/day) in corn oil by gavage 5 days/week for 13 weeks, dose-dependent increases in tissue concentrations were observed for liver, adipose tissue, skin, and blood (DeVito et al. 1998). After 13 weeks of treatment, tissue concentrations of CDDs were highest in liver, followed by adipose tissue, skin, and blood. The study authors suggested that high liver concentrations of CDDs are consistent with an inducible hepatic binding protein for dioxin-like compounds. Liver and adipose levels of CDDs were monitored in male Sprague-Dawley rats fed diets containing a mixture of CDDs (TCDD, PeCDD, HxCDD, HpCDD, and OCDD) from contaminated milk for 120 days (Laurent et al. 2005). Liver and adipose tissue levels of CDDs remained constant after approximately 1.5 months, with greater amounts found in the liver compared to adipose tissue; the ratio of liver:adipose tissue CDD levels ranged from 2.5 for 2,3,7,8-TCDD to 33 for OCDD. Intermediate-duration exposure to 2,3,7,8-TCDD in the feed has been shown to produce higher liver accumulation in male rats (85%) than in female rats (70%) (Fries and Marrow 1975). The percentage retained was related to intake, and at steady state, the total amount retained was about 10.5 times the average daily intake.

Intermediate-duration studies have also been conducted with radioactively labeled OCDD. OCDD had similar patterns of distribution and similar half-lives as 2,3,7,8-TCDD in Sprague-Dawley (Norback et al. 1975) and Fischer-344 rats (Birnbaum and Couture 1988; Birnbaum et al. 1989a). Most of the absorbed amount (50–97%) was found in the liver and was associated with the microsomal fractions. Skin and adipose-tissue levels were much lower. Radioactivity was also detected in the kidneys, heart, testes, skeletal muscle, and serum.

Dermal Exposure. Male Fischer-344 rats absorbed 40% of a single dermal dose of 0.32 µg of radioactive 2,3,7,8-TCDD/kg over a period of 120 hours after dosing (Banks and Birnbaum 1991). The major depots for 2,3,7,8-TCDD-derived radioactivity were the liver and adipose tissue. Seventy-two hours after dosing, the liver and adipose tissue retained approximately 21 and 8% of the administered dose, respectively. Distribution to the liver increased significantly between 4 and 8 hours and between 12 and 72 hours after dosing. Distribution in fat increased significantly between 12 and 120 hours after dosing. Skin and muscle accumulated considerably less 2,3,7,8-TCDD-derived radioactivity than liver and fat. Within 120 hours of dosing, <4% of the administered dose was found in the skin or muscle tissues. When 2,3,7,8-TCDD was dermally applied to HRS/J hairless mice for an intermediate duration, about 5–6% of the total administered dose (0.0025–0.01 µg/kg, 2 days/week, for 20 weeks) was detected in the liver (Hebert et al. 1990).

3.1.3 Metabolism

Little data were located regarding metabolic pathways of CDDs in humans. However, there is some evidence that 2,3,7,8-TCDD is partially excreted in the feces in the form of metabolites (Sorg et al. 2009; Wendling et al. 1990). Two main metabolites, 2,3,7-trichloro-8-hydroxydibenzo-*p*-dioxin and 1,3,7,8-tetrachloro-2-hydroxydibenzo-*p*-dioxin, were identified in feces, urine, and blood serum of an individual poisoned with TCDD (Sorg et al. 2009). The patient's blood serum level of TCDD was 108,000 pg/g lipid 3 months after the poisoning. Results of an *in vitro* study using recombinant yeast microsomes containing human CYP isozymes from human liver show that 2,3,7-TrCDD undergoes sequential metabolism by CYP and UDP-glucuronosyltransferase (Kasai et al. 2004). Using the same *in vitro* model, several mono-, di-, and tri-CDDs have been shown to be metabolized by multiple forms of CYP (Inouye et al. 2002). Metabolites included products of multiple reactions, including several types of hydroxylation reactions. Enzymes CYP1A1 and CYP1A2 exhibited the highest activity for mono-, di-, and tri-CDDs, although other CYP isozymes (PYP2C8, CYP2C9, and CYP3A4) did not show any significant activity for CDDs; none of the CYP isozymes showed any activity toward 2,3,7,8-TCDD.

A study in animals indicates that 2,3,7,8-TCDD is metabolized slowly in mammals (Koshakji et al. 1984). Metabolic transformation by phase I metabolizing enzymes includes oxidation and reductive dechlorination, as well as oxygen bridge cleavage. This is followed by conjugation reactions catalyzed by phase II type enzymes, which facilitate excretion by adding more polar groups to the molecule. A study in guinea pigs showed that only 28% of the radioactivity in the tissues 45 days following exposure to

[^{3}H]-2,3,7,8-TCDD was in the form of metabolites (Olson 1986). Results from high performance liquid chromatography (HPLC) suggested the presence of at least five [^{3}H]-labeled metabolites of 2,3,7,8-TCDD, but their structure was not established. The results indicated that in the guinea pig, the metabolites of 2,3,7,8-TCDD may not leave the body rapidly. In rats and hamsters, metabolism appears to be required for urinary and biliary excretion (Olson et al. 1980a). Metabolites of 2,3,7,8-TCDD are not generally detected in tissues, suggesting that for most species, 2,3,7,8-TCDD is readily eliminated following metabolism.

The role of CYP1A2 in the overall metabolism of CDDs has been studied in *CYP1A2* knockout mice (lacking the *Cyp1a2* gene) following single-dose oral exposure (Hakk and Diliberto 2002, 2003; Hakk et al. 2009). Results show that mice with the *Cyp1a2* gene (wild-type mice) only metabolize slightly more 2,3,7,8-TCDD or 1,2,3,7,8-PeCDD than *Cyp1a2* knockout mice, indicating that sequestration of CDDs by binding to CYP1A2 does not have an important effect on metabolism by other CYP isozymes or other enzymes (Hakk and Diliberto 2002, 2003). Overall metabolism did not exhibit dose-dependence in either wild-type or *CYP1A2* knockout mice (Hakk et al. 2009).

Metabolism of 1,3,6,8-TCDD was studied in hepatic microsomes obtained from male C57BL/6 mice administered a single oral dose of 2,3,7,8-TCDD in corn oil (Aozasa et al. 1996). Metabolites of 1,3,6,8-TCDD included several hydroxylation products, which appear to be further metabolized to other compounds, including quinones, sulfate conjugates, and other smaller compounds (not identified). Metabolites isolated from urine, bile, and feces of Sprague-Dawley rats administered a single dose of [^{14}C]-1,2,7,8-TCDD (8 mg/kg) in corn oil by gavage include hydroxylation products, glucuronide conjugates, and sulfide conjugates (Hakk et al. 2001). Similar metabolic profiles were reported for 1,3,7,8- and 1,4,7,8-TCDD (Huwe et al. 1997, 1998; Petroske et al. 1997).

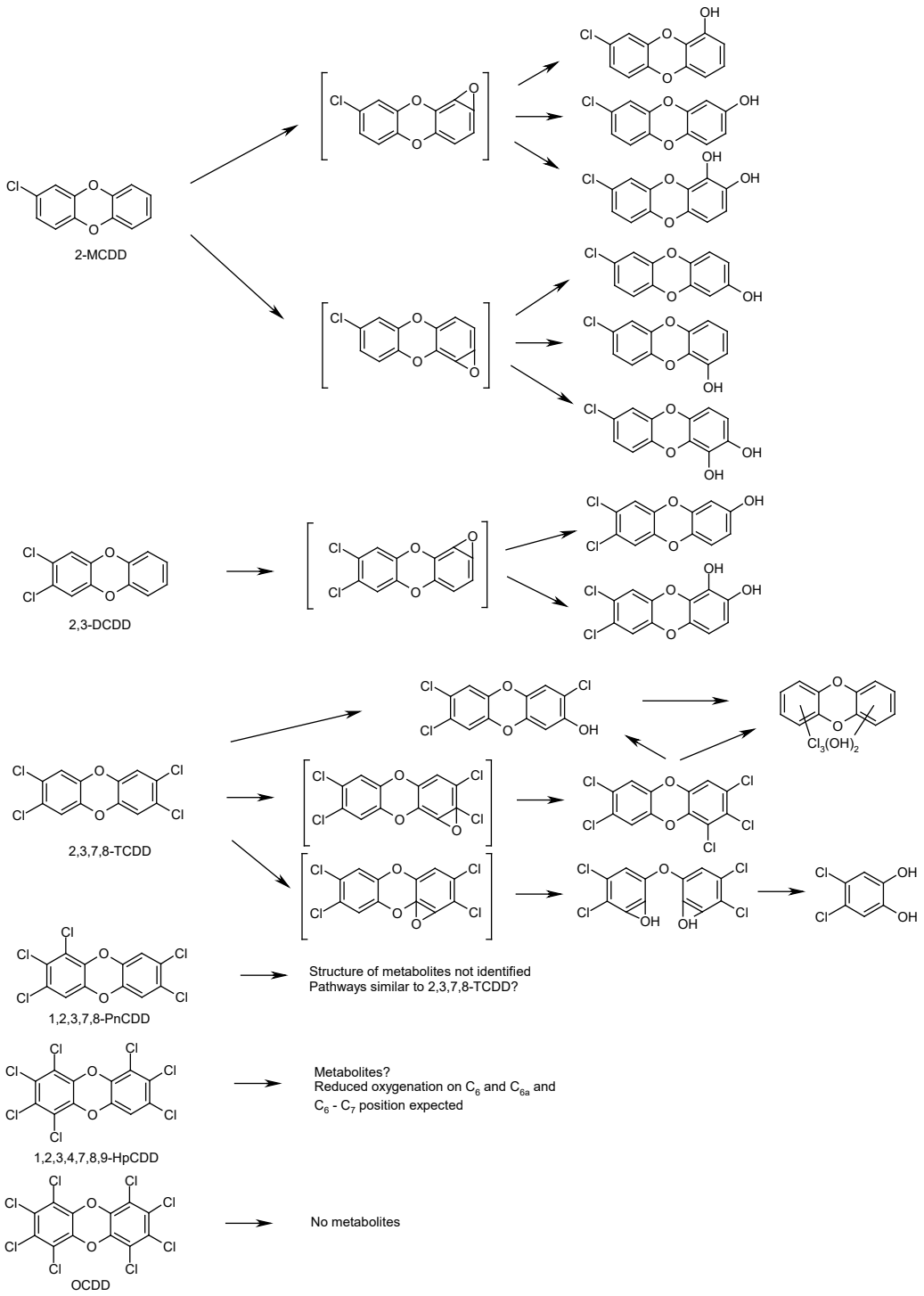
An *in vitro* study with isolated rat hepatocytes identified 1–hydroxy-2,3,7,8-TCDD and 8-hydroxy-2,3,7-TrCDD as metabolites (Sawahata et al. 1982). 2-Hydroxy-1,3,7,8-TCDD was found to be the major metabolite of 2,3,7,8-TCDD in dogs but not in rats (Poiger et al. 1982). The metabolites from dogs administered to rats were eliminated as conjugates in the bile (Weber et al. 1982). Self-induction of 2,3,7,8-TCDD metabolism was reported in both species (Poiger and Schlatter 1985; Weber et al. 1982). A single 10 µg/kg dose of unlabeled 2,3,7,8-TCDD 9 days prior to administration of [^{3}H]-2,3,7,8-TCDD resulted in a doubling of the amount of radioactivity eliminated in the bile of dogs. When the 2,3,7,8-TCDD metabolites, 2-hydroxy-2,3,7-TrCDD and 2-hydroxy-1,3,7,8-TCDD, were synthesized and injected intraperitoneal into Wistar rats, no toxic effects were observed (Mason and Safe 1986). This

supports the observation that the extract from the bile of 2,3,7,8-TCDD-treated dogs is about 100 times less toxic to rats and guinea pigs than pure 2,3,7,8-TCDD (Poiger et al. 1982). The lack of toxicity of the 2,3,7,8-TCDD metabolites suggests that autoinduction of its own metabolism in animals is a detoxification mechanism.

Data regarding other 2,3,7,8-substituted CDDs are limited. Wacker et al. (1986) found at least three phenolic radiolabeled metabolites of [^{14}C]-1,2,3,7,8-PeCDD in rat bile after treatment with glucuronidase and methylation, indicating the probability of formation of hydroxy metabolites. Results from studies in rats revealed no metabolites of OCDD, as expected from the fully chlorinated molecule (Birnbaum and Couture 1988; Tulp and Hutzinger 1978).

CDDs induce both phase I and phase II drug-metabolizing enzymes including AHH, ethoxyresorufin-O-deethylase (EROD), UDP-glucuronosyltransferase, glutathione S-transferase, and DT-diaphorase (Van den Berg et al. 1994). These enzymes are responsible for the metabolism of a variety of exogenous and endogenous substances. Pretreatment of C57BL/6J mice with 2,3,7,8-TCDD increased hepatic accumulation of a subsequent radiolabeled dose (total liver burden increased about 50%), whereas distribution to the kidney, fat, heart, lung, and gastrointestinal tract were reciprocally decreased (Curtis et al. 1990). The data indicated that an inducible, saturable system is involved in 2,3,7,8-TCDD toxicokinetics. The pretreatment, however, did not alter the hepatic metabolism of 2,3,7,8-TCDD in exposed mice. Similarly, the rate of metabolism of 2,3,7,8-TCDD in hepatocytes from 2,3,7,8-TCDD-pretreated (induced) guinea pigs and mice was unchanged from that in untreated animals (Olson and Wroblewski 1985; Shen et al. 1989; Wroblewski and Olson 1985). In contrast, the rate of metabolism in hepatocytes from 2,3,7,8-TCDD-pretreated rats was 3.2-fold greater than the rate in hepatocytes from control rats and about 9 times greater than in hepatocytes from 2,3,7,8-TCDD-pretreated guinea pigs. The difference between the 2,3,7,8-TCDD ability to induce its own rate of metabolism in rats and guinea pigs could be a factor in the difference between the susceptibility to 2,3,7,8-TCDD-induced toxicity in these two species, because the parent compound rather than metabolites is the toxic agent (Poland and Glover 1979). A generalized scheme of metabolic pathways for CDDs based on information from *in vivo* mammalian studies was proposed by Van den Berg et al. (1994) and is presented in Figure 3-1.

Figure 3-1. A Generalized Scheme of Pathways for the Biotransformation of CDDs Based on Information from *In Vivo* Mammalian Studies



CDD = chlorinated dibenzo-*p*-dioxin; DCDD = dichlorodibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; MCDD = monochlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin; PnCDD = pentachlorodibenzo-*p*-dioxin; TCDD = tetrachlorodibenzo-*p*-dioxin

Source: Van der Berg et al. 1994

3.1.4 Excretion

In humans, the primary route of excretion of absorbed CDDs is the feces (Rohde et al. 1999; Schlummer et al. 1998). Results of a study in two female patients with severe TCDD intoxication show that 2,3,7,8-TCDD undergoes cutaneous elimination (Geusau et al. 2001a). The TCDD exposure source for these patients is unknown (Geusau et al. 2001b). Cutaneous elimination was approximately 1–2% of the total daily TCDD elimination, when adjusted for skin surface area.

A median half-life of 7.1 years was estimated for 2,3,7,8-TCDD in a group of 36 Vietnam veterans (CDC 1987; Pirkle et al. 1989). The calculation was based on the decrease of 2,3,7,8-TCDD serum levels that were measured in these individuals in 1982 and again in 1987. The individual half-life values varied from 2.9 to 26.9 years. In an expanded half-life study of 343 Vietnam veterans participating in Operation Ranch Hand, which included the subjects of the Pirkle et al. (1989) study, a half-life estimate of 8.7 years (95% CI: 8.0–9.5 years) was calculated (Michalek et al. 1996). The half-life estimate was calculated using 2,3,7,8-TCDD levels in blood samples collected in 1982, 1987, and 1992. An earlier study of these subjects (Wolfe et al. 1994), which used data from two blood collection periods (1982 and 1987), estimated a half-life of 11.3 years (95% CI=10–14.1 years). This half-life of 11.3 years was considered too high because it was based on restricted analysis of veterans with 2,3,7,8-TCDD levels >10 ppt. By conditioning the data to lie above a line with slope equal to the negative of the decay rate, the analysis yielded a revised half-life of 8.7 years. In a 15-year follow-up of 97 Operation Range Hand veterans, Michalek and Tripathi (1999) estimated a half-life of 7.6 years (95% CI: 7.0–8.2 years). Michalek et al. (2002) conducted a combined analysis of Seveso adults and Operation Ranch Hand veterans. In the Seveso cohort, a period of fast elimination (half-life: 0.34 years) during the first 0.27 years after exposure was followed by a period of slower elimination (half-life: 6.9 years) from 3 to 16.35 years. In the Ranch Hand cohort, the half-life from 9 to 33 years (7.5 years) was similar to that of the Seveso population. The study authors noted that results in the Seveso cohort are consistent with a two-compartment model, with a distribution phase with rapid elimination, followed by a slower elimination phase.

Several other studies have calculated 2,3,7,8-TCDD half-lives. A mean half-life of 5.8 years was estimated from repeated samples from 29 BASF AG facility workers with initial 2,3,7,8-TCDD serum lipid concentrations of 29–553 ppt (Ott and Zober 1996). In a study of 48 German workers at a pesticide facility who were exposed to a mixture of CDDs/CDFs, a median half-life of 7.2 years was estimated for 2,3,7,8-TCDD (Flesch-Janys et al. 1996). Needham et al. (1994) estimated a half-life of 8.2 years in 27 Seveso residents with initial serum 2,3,7,8-TCDD levels of 130–3,830 ppt. A study of Seveso women

found the half-life to vary with the age at time of exposure (Warner et al. 2014). The half-lives were 7.1 years in women who were exposed at >10 years of age, 5.2 years in women who were exposed at 6–10 years of age, and 4.3 years in women who were <5 years of age at the time of exposure. Using data from a human subject ingesting a single dose of 1.14 ng/kg 2,3,7,8-TCDD, Poiger and Schlatter (1986) calculated a half-life of 2,120 days (5.8 years). Geyer et al. (1986) noted that they calculated a half-life of 3.5–6.9 years, but did not describe the basis of this estimation. Overall, there is good agreement between the 2,3,7,8-TCDD half-lives estimated in four different populations (Vietnam veterans, BASF AG cohort, German pesticide workers, and Seveso residents); the half-lives were 5.8–8.7 years (Aylward et al. 2013; Flesch-Janys et al. 1996; Michalek et al. 1996; Needham et al. 1994; Ott and Zober 1996; Yamamoto et al. 2015b). Several studies have found correlations between percentage of body fat and 2,3,7,8-TCDD elimination half-times (Flesch-Janys et al. 1996; Michalek et al. 1996; Ott and Zober 1996; Wolfe et al. 1994). Ott and Zober (1996) estimated half-lives of 5.1 and 8.9 years in subjects with 20 and 30% body fat, respectively. Age and body burden also appear to influence 2,3,7,8-TCDD half-life (Kerger et al. 2006). Among Seveso children (<18 years of age at the time of the accident), half-lives of 2.4 and 1.6 years were estimated for children with 2,3,7,8-TCDD levels <700 ppt (average concentration of 219 ppt) and >700 ppt (average concentration of 1,400 ppt), respectively; the half-lives were significantly different. Similarly, the half-life in children <18 years of age at the final sampling was 1.6 years, which was significantly lower than the half-life of 3.2 years in children ≥18 years of age at the final sampling.

There are limited data available on the elimination of other CDD congeners in humans. In the Flesch-Janys et al. (1996) study of 48 workers at a German pesticide facility, elimination half-times were estimated for several CDD congeners. The estimated half-lives were 15.7 years for 1,2,3,7,8-PeCDD, 8.4 years for 1,2,3,4,7,8-HxCDD, 13.1 years for 1,2,3,6,7,8-HxCDD, 4.9 years for 1,2,3,7,8,9-HxCDD, 3.7 years for 1,2,3,4,6,7,8-HpCDD, and 6.7 years for OCDD. In a study of six German workers with high CDD/CDF body burdens, elimination half-lives corrected for alterations in body weight ranged from 3.5 years for 1,2,3,4,6,7,8-HpCDF to 7.9 years for 2,3,7,8-TCDD and 15 years for 1,2,3,4,7,8-HxCDD (Rohde et al. 1997). In the same study, half-lives for elimination due only to fecal excretion ranged from 10 years for OCDD to 22 years for 2,3,7,8-TCDD to 27 years for 1,2,3,7,8-PeCDD. The half-lives for 2,3,4,7,8-PeCDF in humans exposed to contaminated rice oil in the Yusho incident ranged from 2 to 30 years, and were inversely dependent on adipose tissue concentrations above approximately 10 ng/kg body weight (i.e., the higher the body burden, the faster the elimination) (Ryan et al. 1993a). Aylward et al. (2013) estimated elimination half-lives for CDD congeners and TEQ in former workers (n=56) at a chlorophenol plant. Median intrinsic half-lives (body burden half-life adjusted for continued exposure) were as follows: 10.7 years for PeCDD (specific congener not reported), 7.0 years for

1,2,3,4,7,8-HxCDD, 9.0 years for 1,2,3,6,7,8-HxCDD; 6.3 years for 1,2,3,7,8,9-HxCDD, 6.7 years for 1,2,3,4,7,8,9-HpCDD, 7.3 years for OCDD, and 8.7 years for total TEQ (CDDs, CDFs, and dioxin-like PCBs. Yamamoto et al. (2015b) estimated the following elimination half-lives for CDD congeners in former workers (n=16) at an incineration plant measured over a 7-year period beginning 3 years after the plant was shut down. Mean half-lives were as follows: 13.8 years for 1,2,3,7,8-PeCDD, 10.0 years for 1,2,3,4,7,8-HxCDD, 12.5 years for 1,2,3,6,7,8-HxCDD, 4.8 years for 1,2,3,7,8,9-HxCDD, 6.7 years for 1,2,3,4,7,8,9-HpCDD, and 9.1 years for TEQ (CDDs, CDFs, and dioxin-like PCBs).

Inhalation Exposure. In male Fischer-344 rats administered a single intratracheal dose of 0.32 μg labeled 2,3,7,8-TCDD/kg, fecal elimination was the major route of elimination over a 3-day period after dosing (Diliberto et al. 1996). The cumulative fecal excretion of 26.3% of the administered dose was observed over 3 days following exposure. Approximately 4% of the dose was excreted in the feces on day 3. The cumulative urinary excretion was only 1.3% of the administered dose.

Oral Exposure. Elimination across the gastrointestinal tract is an important elimination pathway for absorbed CDDs in humans. Results of a mass balance study in six men with high body burdens of CDDs showed that fecal elimination of 2,3,7,8-TCDD and OCDD was 37 and 90%, respectively, of total elimination (Rohde et al. 1999). Fecal elimination of CDDs exceeded dietary intake, indicating gastrointestinal excretion of CDDs from diet or body stores. Similar results were reported in a mass balance study in 14 volunteers (7 males and 7 females), with fecal excretion exceeding dietary intake by approximately 2-fold (Schrey et al. 1998). Fecal excretion was the main route of elimination also in a patient poisoned with a high level of TCDD (Sorg et al. 2009). During the 3-year period of follow-up testing, the patient eliminated in feces and urine the total amount of the two major metabolites equivalent to about 95 μg of TCDD (i.e., 38% of total TCDD eliminated by all means). The half-life of TCDD in this patient was 15.4 months. The elimination pattern fits into a model predicting that expected half-life of TCDD ranges from <3 years in people exposed to high levels (>10,000 pg/g serum lipids of internal dose) to >10 years in those exposed to lower levels (<50 pg/g serum lipids of internal dose) (Aylward et al. 2005).

The half-life for elimination of a single oral dose of 1.14 ng/kg [^{3}H]-2,3,7,8-TCDD in a volunteer was calculated as 5.8 years (Poiger and Schlatter 1986). The excretion in feces was high during the first few days (up to day 6) probably because of elimination of unabsorbed material. During these first few days, about 12% of the administered dose was excreted. However, during days 7–125, only about 3.5%

of the administered dose was eliminated. Urinary levels of radioactivity did not exceed the background levels.

Studies in animals have shown that 2,3,7,8-TCDD can be excreted in feces and urine. In C57BL/6N and *CYP1A2* knockout mice administered single oral doses of [^{3}H]-2,3,7,8-TCDD (0.1 or 10 μg/kg), 24–31 and <5% of the administered dose were eliminated in feces and urine, respectively, within 4 days; no dose-related differences in excretion were observed (Hakk et al. 2009). Following oral administration of [^{14}C]-2,3,7,8-TCDD (1.25 mg/kg) to male Sprague-Dawley rats, only 0.27% of the administered dose of [^{14}C] was eliminated in urine within 3 days (Hakk et al. 1998). Of the [^{14}C] excreted in urine, approximately 8.8% of the urinary [^{14}C] was bound to albumin and approximately 64.2% was unbound. In bile-duct cannulated rats, 9.6% of [^{14}C] in bile was bound to an unidentified 89kDa protein and 76.6% was unbound. In male Sprague-Dawley rats administered a single dose of [^{14}C]-1,2,7,8-TCDD (8 mg/kg) in peanut oil by gavage, 94.2% of the administered radioactivity was recovered in feces (79.8%) and urine (14.3%).

Biliary excretion was estimated as 32.4%, using biliary-cannulated rats (Hakk et al. 2001). Fecal excretion is also the predominant elimination pathway for 1,4,7,8-TCDD (Huwe et al. 1998). In male Sprague-Dawley rats administered a single dose of [^{14}C]-1,4,7,8-TCDD (2 mg total dose), 88.8 and 3.3% of the administered [^{14}C] was eliminated in feces and urine, respectively, within 3 days; biliary excretion was estimated as >30% of the administered [^{14}C]. Following oral administration of [^{14}C]-1,2,3,7,8-PeCDD (2.9 mg/kg) to male Sprague-Dawley rats, only 0.22% of the administered dose of [^{14}C] was eliminated in urine within 3 days (Hakk et al. 1999). Of the [^{14}C] excreted in urine, approximately 17.9% of the urinary [^{14}C] was bound to albumin and approximately 78.1% was unbound. In bile-duct cannulated rats, 7.2% of [^{14}C] in bile was bound to an unidentified 89kDa protein and 91.1% was unbound.

Studies in animals indicated that elimination of 2,3,7,8-TCDD is a relatively slow process. However, the results showed a great variability among species. The half-life for 2,3,7,8-TCDD elimination was 14.95 days in Syrian hamsters (Olson et al. 1980b), 12 and 14 days in male and female Sprague-Dawley rats, respectively (Fries and Marrow 1975), 17 days in male Sprague-Dawley rats in another study (Piper et al. 1973), and 94 days in guinea pigs, the most sensitive species to the acute toxicity of 2,3,7,8-TCDD (Olson 1986). In contrast, the elimination half-life was 391 days in monkeys chronically exposed to low doses of 2,3,7,8-TCDD in the feed (Bowman et al. 1989b). A similar half-life of about 1 year was observed in monkeys after a single-dose exposure (McNulty et al. 1982). In addition, studies of

2,3,7,8-TCDD half-life in highly exposed rats (Abraham et al. 1988), Rhesus monkeys (McNulty et al. 1982), and marmoset monkeys showed that rates of excretion decreased with dose. In mice, the blood elimination half-life is affected by obesity. Obesity in C57BL/6J mice resulted in a 2- and 10-fold increase in the blood elimination half-life following an oral dose of [^{3}H]-labeled 2,3,7,8-TCDD (5 or 0.1 μg/kg dose, respectively) (Emond et al. 2018). The clearance of radioactivity after oral exposure to labeled 2,3,7,8-TCDD followed first-order kinetics in most studies. Fecal elimination was the major route, although excretion in urine, expired air, and milk was also reported.

When Sprague-Dawley rats were given radioactively labeled 2,3,7,8-TCDD, a total of 53% of the administered radioactivity was excreted by feces in the first 21 days (Piper et al. 1973). Elimination of 2,3,7,8-TCDD-derived radioactivity in urine and expired air was 13 and 3% of the administered dose, respectively. Over a 3-day period after dosing, 32.2% of a single gavage dose of 0.32 μg of 2,3,7,8-TCDD/kg was eliminated in the feces of male Fischer-344 rats (Diliberto et al. 1996). Only 1.4% of the administered dose was excreted in the urine over the same period. About 20–30% of the total oral 2,3,7,8-TCDD dose was eliminated in the bile of cholecystectomized and cannulated dogs (Poiger et al. 1982). In addition, excretion of unchanged 2,3,7,8-TCDD in milk was demonstrated in NMRI mice (Nau et al. 1986) and monkeys (Bowman et al. 1989b) after oral exposure.

Of the other congeners, several have been studied. An elimination half-life of 29.5 days was estimated for 1,2,3,7,8-PeCDD in Sprague-Dawley rats following a single oral exposure (Wacker et al. 1986). OCDD was more persistent in Fischer-344 rats, with an estimated elimination half-life of 3–5 months following 10 daily oral doses (Birnbaum and Couture 1988). These congeners were excreted primarily in the feces following biliary elimination as metabolites (1,2,3,7,8-PeCDD, at least three phenolic metabolites) or parent compound. A 13-week dosing study in which Sprague-Dawley rats were administered various mixtures of CDDs estimated liver half-lives of 14.5, 29.3, 45.6, and 100 days for 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, and 1,2,3,4,6,7,8-HpCDD, respectively (Viluksela et al. 1998a).

Dermal Exposure. Within 120 hours after dermal administration of 0.32 μg/kg 2,3,7,8-TCDD to the clipped back skin of male Fischer-344 rats, 4% of the administered dose was excreted in the feces and <1% was excreted in the urine (Banks and Birnbaum 1991). The rate of 2,3,7,8-TCDD elimination significantly increased over time.

Transfer of CDDs Through the Placenta and Human Milk. CDDs are lipophilic compounds that can concentrate in maternal milk. Therefore, lactation provides an efficient mechanism for decreasing the body burden of these compounds (Schecter and Gasiewicz 1987a). Analysis of data obtained through NHANES provides support that human milk levels of CDDs generally reflect blood lipid levels of CDDs, although these ratios may vary considerably between individuals and over time (Aylward et al. 2003). In a study of 21 Japanese women, statistically significant correlations have been reported between CDD concentrations (expressed as TEQs) in maternal blood and milk ($r=0.695$; $p=0.0007$), maternal adipose tissue ($r=0.913$; $p<0.0001$), and cord blood ($r=0.759$; $p<0.0001$) (Nakano et al. 2005). In a study of primiparous women in Japan, the ratio of milk CDDs to blood CDDs were 0.57 for 2,3,7,8-TCDD, 0.64 for 1,2,3,7,8-PeCDD, 0.55 for 1,2,3,6,7,8-HxCDD, 0.23 for1,2,3,4,6,7,8-HpCDD, 0.08 for OCDD, and 0.11 for total CDDs (Todaka et al. 2008). Analysis of tissue CDDs obtained from 20 maternal-fetal pairs showed the highest levels of CDDs in perinatal venous serum, followed by placenta, cord serum, and human milk (Wang et al. 2004). Transfer of CDDs from mothers to fetuses and infants was assessed by measuring CDDs in maternal blood, cord blood, placenta, maternal adipose tissue, and milk in 22 Japanese women (Suzuki et al. 2005). Results show that CDD congeners with high TEQs accumulate in the placenta relative to maternal blood and that CDD levels in milk are influenced, in part, by maternal adipose levels.

CDD levels in human milk samples have been measured in many studies. The results from some of the surveys of samples taken from 2000 to 2024 are reported in Table 3-1. In general, the levels in milk decreased with decreasing degree of chlorination from octa- to tetra-CDD. Milk samples from industrial countries tended to have higher CDD levels than those from less developed countries.

Fürst et al. (1989) also found that the levels of CDDs found in the milk of mothers breastfeeding their second child were about 20–30% lower than in those breastfeeding their first child. It was further noted that the highest excretion of CDDs was during the first few weeks after delivery. The sharpest decline was observed with OCDD; its excretion was reduced by half between the first and fifth week of lactation. In contrast, there was no significant decline in total HxCDDs in milk during the first year of lactation. The concentration of 1,2,3,4,6,7,8-HpCDD in milk fat showed a steady decline over the 1-year period, but its levels stayed relatively high. 2,3,7,8-TCDD represented the smallest portion of the total CDDs, and its levels in milk continuously declined over the year of lactation. Levels of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8- and 1,2,3,6,7,8-HxCDD, 1,2,3,4,6,7,8-HpCDD, and OCDD were measured in a mother of twins prior to nursing and after 2 years of nursing (Schecter et al. 1996a). There was a 49.5% decrease in the total amount of CDDs in the lipid fraction of the human milk. 2,3,7,8-TCDD had

Table 3-1. Mean Levels of CDDs in Human Milk (ng/kg Milk Fat)

	2,3,7,8-TCDD	1,2,3,7,8-PeCDD	1,2,3,4,7,8-HxCDD	1,2,3,6,7,8-HxCDD	1,2,3,7,8,9-HxCDD	1,2,3,4,7,8,9-HpCDD	OCDD
Canada (n=298) Rawn et al. 2017	0.45	1.7	1.2	8.1	1.3	8.4	35
France (n=244) Focant et al. 2013	1.09	4.36	1.81	16.15	2.32	16.17	74.97
Hungary[a] (n=22) Vigh et al. 2013	0.28–0.34	0.56–0.74	0.66–0.96	2.17–3.32	0.85–1.12	2.57-–3.71	34.51–48.92
Ireland n=16 Houlihan et al. 2021	0.36	1.1	0.43	2.1	0.52	2.5	20
Ireland (n=11[b]) Pratt et al. 2012	0.61	1.85	0.87	4.76	0.88	8.07	49.8
Italy (n=95) Giovannini et al. 2014	0.70 (median)	2.57 (median)	0.98 (median)	5.55 (median)	0.96 (median)	5.12 (median)	32.34 (median)
Italy (n=39) Abballe et al. 2008	1.11–1.79	2.67-–4.17	1.67–2.55	7.08–12.2	1.64–3.26	11.4–16.5	52.1–68.4
Italy (n= 38) Ulaszewska et al. 2011	0.11–0.13	1.54–1.81	0.71–0.72	3.90–4.57	0.51–0.72	3.08–4.23	25.99–28.29
Germany (n=42) Raab et al. 2008	0.86	2.84	1.68	7.95	1.44	8.62	39.95
Latvia (n=15) Bake et al. 2007	1.335	1.556	0.632	2.626	0.518	3.926	30.128
Slovakia (n=32) Chovancová et al. 2011	0.5–0.7	1.3–2.3	0.5–1.6	2.8–4.3	0.7–1.1	4.2–7.8	20.2–41.9
New Zealand (n=39) Mannetje et al. 2013	0.75	1.57		2.87		5.44	30.53
China (n=158) Sun et al. 2010	0.37–0.76	1.23–1.56	0.23–0.70	1.02–2.06	0.10–0.38	1.20–2.98	15.94–22.53

Table 3-1. Mean Levels of CDDs in Human Milk (ng/kg Milk Fat)

	2,3,7,8-TCDD	1,2,3,7,8-PeCDD	1,2,3,4,7,8-HxCDD	1,2,3,6,7,8-HxCDD	1,2,3,7,8,9-HxCDD	1,2,3,4,7,8,9-HpCDD	OCDD
China (n=74) Shen et al. 2012	0.14-0.19	0.43-0.44	1.05-2.74	0.97-1.99	0.77-2.07	2.30-6.58	16.5-28.5
Japan (n=60) Todaka et al. 2008	0.7	3.2		9.8		5.9	40
Taiwan (n=25) Chen et al. 2018	0.457	1.031	0.493	1.798	0.676	2.76	38.8
Africa[c] (number of samples not reported) UNEP 2023	0.10–1.04	0.30–2.05	0.12–1.14	0.39–7.29	0.28–56.01	1.23–61.97	2.91–323.36
Asia and Pacific Islands[d] (number of samples not reported) UNEP 2023	0.19–1.18	0.43–4.98	0.19–2.81	0.80–12.53	0.32–5.24	1.29–43.98	12.29–250.25
Latin America and Caribbean[e] (number of samples not reported) UNEP 2023	0.23–0.61	0.77–1.75	0.30–0.84	1.17–6.04	0.41–1.62	2.19–17.46	6.32–102.83

[a]Human milk samples collected on days 5, 12, and 84 postpartum.
[b]11 pooled samples from 109 women.
[c]Democratic Republic of the Congo, Egypt, Ethiopia, Ghana, Kenya, Mali, Mauritius, Morocco, Nigeria, Senegal, United Republic of Tanzania, Togo, Tunisia, Uganda, Zambia.
[d]Cambodia, Indonesia, Lao, Mongolia, Philippines, Thailand, Viet Nam, Fiji, Kiribati, Marshall Islands, Niue, Palu, Samoa, Solomon Islands, Tuvalu, Vanuatu.
[e]Antigua and Barbuda, Argentina, Barbados, Brazil, Chile, Colombia, Ecuador, Jamaica, Mexico, Peru, Uruguay.

CDD = chlorinated dibenzo-*p*-dioxin; HpCDD = heptachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzo-*p*-dioxin; OCDD = octachlorodibenzo-*p*-dioxin; TCDD = tetrachlorodibenzo-*p*-dioxin

the largest percent decline in CDD levels, a decrease of 83.9%. A 52.4% decrease in maternal serum lipid levels of total CDD was also observed; the largest percent decline was an 86.8% decline in 1,2,3,7,8-PeCDD levels. In a study of 22 breastfeeding mothers, Vigh et al. (2013) found that human milk concentrations of TEQ (CDDs, CDFs) declined during lactation when concentrations were measured on days 5, 12, and 84 of lactation. The total decrease was from 3.17 to 2.41 pg TEQ/g lipid. In this same study, mean daily intakes of TEQ (CDDs, CDFs plus dioxin-like PCBs) by breastfeeding infants were estimated to be 11.71, 16.54, and 11.59 pg TEQ/kg body weight on days 5, 12, and 84 of lactation, respectively.

Several studies have shown that CDDs in human milk are readily absorbed by nursing infants. In a 19-week-old nursing infant, absorption was estimated as the difference between ingestion and the amount of CDDs found in the feces over a period of 12 days (McLachlan 1993). The mother was 32 years old and nursing for the first time. Several CDD congeners were determined in the milk: 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, three hexachloro-substituted congeners, 1,2,3,4,6,7,8-HpCDD, and OCDD. The percentage of dose absorbed was 90–95%, except for the hepta-substituted congeners and OCDD, which exhibited absorption rates of 61 and 23%, respectively. The percentage of the dose absorbed increased slightly if corrections were made for background levels in the diapers. Similar results were reported by Pluim et al. (1993b) who measured the amount of CDDs consumed via human milk and excreted in the feces in three infants at the ages of 4, 8, and 12 weeks. Because of the high content of CDDs of the diapers relative to the feces, the percentage of dose absorbed was not determined. However, the results showed that, with the exception of OCDD, the bioavailability from human milk was >95%. At 4 weeks of age, the average cumulative intake of CDDs from human milk was 132.1 pg TEQ per kg body weight; 37.4 pg TEQ 2,3,7,8-TCDD, 46.2 pg TEQ 1,2,3,7,8-PeCDD, and 24.4 pg TEQ 1,2,3,6,7,8-HxCDD. With the inclusion of CDFs, the total TEQ at 4 weeks was approximately 257 pg/kg body weight. Exposure to CDDs and CDFs from lactation decreased at 8 and 12 weeks mainly due to a decrease in their concentration in whole human milk, which resulted from a reduced fat content of the milk (the depletion of body burden of the mother while nursing may have also contributed). Abraham et al. (1994, 1996) and Dahl et al. (1995) also reported almost complete absorption of lower chlorinated CDDs and CDFs in breastfed infants during the first year of life. It was also noticed that intake of CDDs and CDFs was up to 50 times higher in breastfed infants compared with a formula-fed infant (Abraham et al. 1996). The latter study further showed that despite much lower intake of CDDs and CDFs after weaning, the concentration of these compounds in stool fat did not decrease substantially, suggesting that concentration in fecal fat more or less reflect that in body fat. Also, at 11 months of age, TEQ concentrations in blood from

formula-fed infants were <25% of maternal values and about 10 times lower than in infants breastfed for 6–7 months (Abraham et al. 1996).

Transplacental transfer of CDDs and CDFs has been demonstrated in humans and animal models. Total TEQ (CDDs plus CDFs) concentrations in maternal and cord plasma (measured using a chemical activated luciferase expression bioassay, CALUX) were similar and correlated when measured just prior to delivery (Pedersen et al. 2010). Schecter et al. (1996b) presented data on the levels of CDDs and CDFs in human fetal tissues (8–14 weeks gestational age with placenta removed) and in placentas from women from the general population who had normal deliveries. On a lipid basis, the total TEQ (CDDs plus CDFs) in a pool of 14 placentas was 10.1 ng/kg; half of this amount (5.3 ng/kg) was measured in a pool of fetal tissues from 10 fetuses. In an analysis of 43 samples of human milk, Schecter et al. (1996b) found that the total concentration of CDDs and CDFs was 16.7 ng/kg (expressed as TEQ). The study authors also calculated that the TEQ body burden for the pooled fetal tissue was 0.034 ng/kg body weight; for pooled placentas, they calculated a total TEQ of 0.086 ng/kg wet weight. These results suggest that the transfer of CDDs to the fetus may be somewhat limited. *In vitro* vascular perfusion of human placental tissue with 2,3,7,8-TCDD resulted in accumulation of 2,3,7,8-TCDD in the placental tissue (Pedersen et al. 2010).

The influence of maternal transfer (placental and via human milk) of CDDs/CDFs on the body burden of newborns and infants was further investigated by Kreuzer et al. (1997). These investigators also developed a pharmacokinetic model for 2,3,7,8-TCDD that allowed them to simulate body and tissue burden for the entire human lifetime as a function of 2,3,7,8-TCDD uptake from contaminated nutrition. On a lipid basis, the concentrations of 2,3,7,8-TCDD in adipose tissue and liver of breastfed infants who died of sudden infant death syndrome were 0.4–4 and 0.5–4 ppt, respectively. The corresponding values in non-breastfed infants were 0.2–0.8 and 0.3–0.7 ppt. Similar values were detected in adipose tissue and livers of three stillborn babies, confirming the placental transfer of these chemicals to the fetus. The model developed by Kreuzer et al. (1997) reflected sex- and age-dependent changes in body weight, volumes of liver, adipose and muscle tissue, food consumption, and excretion of feces and was used to predict the half-life of elimination of 2,3,7,8-TCDD and its concentrations in adipose tissue, blood, liver, and feces at different ages. Also, the influence of breastfeeding on the 2,3,7,8-TCDD burden of the mother, her milk, and her child was simulated. The study authors used their own data, as well as those from others, to validate the model. For non-breastfed infants, the model predicted a decrease in the concentration of 2,3,7,8-TCDD in lipids during the first year, and this was supported by the empirical data. For infants exclusively breastfed, the model predicted an increase in 2,3,7,8-TCDD burden

followed by a decrease after weaning, and this was also confirmed by the measured data. Model validation of 2,3,7,8-TCDD concentrations in liver for the 20 infants investigated and in adipose tissue, blood, and feces for data in infants published by others showed good agreement between the simulated and experimental values. Since one of the model's assumptions was that the concentration of 2,3,7,8-TCDD in fecal lipids reflected the concentration in lipids of the organism, the good correlation between predicted and empirical data validated the assumption. Under the assumption that the 2,3,7,8-TCDD concentration in lipids of human milk equals the concentration in the maternal organism, the model predicted a value of 2.23 ng 2,3,7,8-TCDD/kg lipid for the beginning of the nursing period. The model further predicted that the concentration of 2,3,7,8-TCDD in milk decreases with duration of breastfeeding, such that after 6 months of daily nursing, the concentration in milk and maternal body lipids would be approximately 70% of the value at the time of delivery. These predictions were in good agreement with published values. Lastly, the investigators modeled the concentration of 2,3,7,8-TCDD in lipids or blood of a male subject for a time span of 60 years and compared it with literature values for German subjects. One of two curves constructed was computed assuming breastfeeding for the first 6 months of life followed by formula up to 1 year and the other considering feeding only formula for the same period of time. In both cases, further nutrition was simulated to consist of the common diet. The predicted curves differed considerably during the first years of life. For the non-breastfed case, 2,3,7,8-TCDD concentrations decreased during the first year and subsequently increased, reaching a maximum at 16 years. For the breastfed case, the simulation yielded a rapid rise of 2,3,7,8-TCDD in lipids followed by a 3-year decrease after weaning and merging at about 7 years with the concentrations of non-breastfed individuals. Subsequently, 2,3,7,8-TCDD concentrations leveled at between 2 and 3 ng 2,3,7,8-TCDD/kg body lipids until the end of life. The latter value agreed with average background levels for the German population. The half-life of nonmetabolic elimination (unchanged 2,3,7,8-TCDD) was calculated to be 0.42 years in newborns and 9.5 years in 40-year-old adults. The half-life of the fraction metabolized by the liver ranged from 1.5 years for newborns to approximately 10 years for a 40-year-old individual. The 3 times greater elimination half-life for the metabolized fraction relative to the nonmetabolized fraction in infants suggests that metabolic elimination does not play a major role in the elimination of 2,3,7,8-TCDD in infants. A key finding from the Kreuzer et al. (1997) study is the model prediction that the increased 2,3,7,8-TCDD burden observed as a result of breastfeeding does not lead to a raised lifetime value.

Maternal-fetal transfer has been evaluated in numerous studies in several animal models; results show that placental-fetal transfer is much lower than fetal transfer through lactation. However, maternal-fetal transfer during sensitive periods of organogenesis is biologically important as evidenced by effects on

fetuses or offspring exposed *in utero*. One day following an oral dose of [^{14}C]-labeled 2,3,7,8-TCDD 10 μg/kg administered late in pregnancy, radiolabel recovered in fetuses was 0.02% of the administered dose (Ishida et al. 2010). In this same study, the highest concentration of radiolabel was found in fetal liver while fetal brain contained approximately 20–25% of the concentration in liver. Following a single dose of [^{3}H]-labeled 2,3,7,8-TCDD administered to Long-Evans rats late in pregnancy, the highest concentrations of radioactivity in offspring were found in liver and adipose on PNDs 49, 69, and 70 (Yonemoto et al. 2005). Following administration of single oral doses of several CDDs to Long-Evans rats on GD 15, only 0.5–3% of the administered dose was transferred to the fetus, compared to postnatal transfer of 7–28% through milk (Chen et al. 2001). Following oral administration of a single dose of [^{3}H]-2,3,7,8-TCDD (1.15 μg/kg in corn oil) to pregnant Long-Evans rats on GD 8, the amount of TCDD transferred to the fetus increased from 0.12 to 0.21% of the administered dose from GD 9 to 16 (Hurst et al. 1998a). Similar fetal tissue TCDD levels were observed on GD 21 following exposure of pregnant Long-Evans rats to [^{3}H]-2,3,7,8-TCDD on GD 8 or 15 (Hurst et al. 1998b). Following administration of single oral doses of [^{3}H]-2,3,7,8-TCDD (1, 10, or 30 mg/kg in corn oil) to pregnant Long-Evan rats (1 μg/kg in corn oil) on GD 15, fetal tissues concentration of TCDD were significantly and highly correlated with TCDD concentrations in maternal blood ($r=0.932$; $p<0.0001$) (Hurst et al. 2000a). Fetal tissue concentrations of pups born to female Long-Evans rats administered [^{3}H]-2,3,7,8-TCDD (1, 10, or 30 mg/kg in corn oil) 5 days/week for 13 weeks prior to mating and throughout gestation were similar to those observed following administration of single doses of [^{3}H]-2,3,7,8-TCDD (1, 10, or 30 mg/kg in corn oil) on GD 8 or 15 (Hurst et al. 2000b). Levels of CDDs in placenta exhibit a dose-dependent decrease, possibly due to increased maternal sequestration in the liver due to induction of CYP1A2 (Chen et al. 2000). In fetal liver, a dose-dependent increase in liver:fat TCDD levels, consistent with a similar hepatic sequestration CYP1A2 mechanism, may exist (Yonemoto et al. 2005).

Excretion into milk represents a major pathway for maternal elimination of CDDs and, therefore, for exposure to offspring. In C57BL/6N mice administered a single oral dose of 30 μg [^{14}C]-2,3,7,8-TCDD/kg on GD 11, the levels of 2,3,7,8-TCDD-derived radioactivity in the embryos on GD 12, 13, or 14 were <0.5% of the total 2,3,7,8-TCDD dose (Weber and Birnbaum 1985). In the dams, the highest concentration of radioactivity was in the liver (50–67% of total dose), whereas embryos had a relatively higher concentration of radioactivity in the heads than in the rest of the body. Approximately 0.03% of the administered dose was delivered to each embryo. In a different study in NMRI mice, pregnant females were administered a single dose of 25 μg [^{14}C]-2,3,7,8-TCDD (oral, intraperitoneal, or subcutaneous) on GD 16 and the distribution of radioactivity was examined in the pups on PNDs 7–36

(Nau et al. 1986). At all times, the highest concentration of radioactivity in the pups (per gram of tissue) was found in the liver; extrahepatic tissues such as intestines and skin had a concentration of radioactivity that was approximately one order of magnitude lower than the liver. During the first postnatal week, 2,3,7,8-TCDD concentrations increased considerably in the pups. It was also found that during the first 2 weeks, the pups received doses of 2,3,7,8-TCDD through milk that were, on a body weight basis, similar to those which had been administered to their mothers prior to birth. In pups raised by untreated foster mothers, 2,3,7,8-TCDD tissue concentrations decreased rapidly due to organ growth with concomitant dilution of 2,3,7,8-TCDD. Abbott et al. (1996) examined the distribution of 2,3,7,8-TCDD in embryonic tissues of mice at times earlier than previous studies. Pregnant mice were treated with 2,3,7,8-TCDD on GD 12 and embryonic tissues were examined at various times from 0.5 to 24 hours after dosing. The rate of accumulation of 2,3,7,8-TCDD reached a maximum in placental tissue in about 3 hours and, following a slight decline, remained relatively constant between 8 and 24 hours. After 24 hours, 0.27% of the maternal dose was detected in the placenta. In embryonic liver, 2,3,7,8-TCDD peaked approximately 8 hours after dosing and decreased thereafter, as opposed to maternal liver, where it remained constant after achieving an apparent maximum. The relative decrease in the rate of concentration in the embryonic liver was attributed to a rapid growth of the tissue during that time period. Distribution of 2,3,7,8-TCDD to embryonic palates followed a pattern similar to that in embryonic liver. Twenty-four hours after dosing, the secondary palates had 0.0045% of the administered maternal dose.

Van den Berg et al. (1987b) examined the transfer of CDDs and CDFs through the placenta and via the milk in Wistar rats. Prenatal exposure of the fetus was studied by administering a diet containing a fly ash extract from a municipal incinerator to rats from day 8 until 17 of pregnancy, after which time the rats were sacrificed. Postnatal transfer was assessed in rats fed the same diet during the first 10 days after delivery while nursing their offspring. Of the 49 tetra- to octa-CDDs, only 7 CDD congeners were detected and all had a 2,3,7,8-chlorine-substitution pattern. In the fetus, 2,3,7,8-TCDD had the highest retention (0.13% of total dose, 0.0092% of the dose/g). Retention decreased with the number of chlorine atoms; HpCDDs and OCDD were not detected. In the liver of offspring, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and the three 2,3,7,8-substituted HxCDDs had the highest retention (5.3–8.1% of total dose, 0.74–1.13% of dose/g). The 2,3,7,8-penta- and hexa-substituted congeners had the highest retention in the livers of pregnant and lactating rats (53.9–80.2% of total dose, 2.9–5.2% of dose/g). No significant differences were found in liver retention of tetra- to octa-chlorinated congeners between pregnant and lactating rats, but lactating females stored less CDDs in their adipose tissue. Similar results were reported by Li et al. (1995c) in Sprague-Dawley rats. The study authors administered a single intravenous dose of 5.6 μg [^{14}C]-2,3,7,8-TCDD/kg to pregnant rats on GD 18. Sacrifices were conducted on GDs 19 and 20,

and PNDs 1 and 5. Groups of neonates were also cross-fostered between treated and nontreated dams to differentially assess transfer of 2,3,7,8-TCDD through the placenta and through nursing. Only about 0.01% of the dose administered to the dams was found in whole livers of fetuses 1 and 2 days after dosing (0.04 and 0.07% of dose/g fetal liver), indicating limited placental transfer. In contrast, the concentration of 2,3,7,8-TCDD in the liver of neonates after 1 day of lactation was 0.65% of the administered dose/g liver, and this increased to 2.88% after 4 days of nursing. Four days after nursing, the liver concentration of 2,3,7,8-TCDD in neonates from dams dosed 1 day after parturition was 4.1% of the administered dose/g of liver, and this was higher than in the dam's liver (3.32%). As in earlier studies, the results from the cross-fostering experiments confirmed that nursing is a major pathway for transfer of 2,3,7,8-TCDD to the offspring.

The transfer of CDDs and CDFs via placenta and through milk was also investigated in a marmoset monkey administered a defined mixture of CDDs and CDFs subcutaneously 11 weeks prior to delivery (Hagenmaier et al. 1990). Concentrations of CDDs and CDFs were measured in a newborn 1 day after birth and in an infant of the same litter after a period of 33 days of lactation. The highest deposition in newborn liver was observed for 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD (54 and 51 pg/g wet weight, respectively) and corresponded to about 0.15% of the administered dose/g tissue. The concentration of all other congeners was <10% of the corresponding concentrations in adults. In contrast to liver, the concentrations of 2,3,7,8-substituted CDDs in newborn adipose tissue were at least one-third the levels in adults, and for OCDD, the concentration in adipose tissue was 3 times higher than in adult adipose tissue. Transfer of CDDs through milk was considerable, though selective. The concentration of 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD in the infant's liver was 395 and 611 pg/g wet tissue, respectively; the corresponding concentrations in the mother's liver were 107 and 326 pg/g. However, the concentration of OCDD in infant's liver was <10% that of the mother's liver. Bowman et al. (1989b) examined the transfer of 2,3,7,8-TCDD from mother to offspring in rhesus monkeys. Female monkeys had been exposed to 2,3,7,8-TCDD for about 4 years to a diet (5 or 25 ppt) that provided an estimated 0.0001–0.0006 μg 2,3,7,8-TCDD/kg/day before breeding. Breeding started 10 months after exposure ceased. At weaning (4 months), the offspring had a concentration of 2,3,7,8-TCDD in mesenteric fat 4.3 times higher than in subcutaneous fat from their respective mothers. Bowman et al. (1989b) estimated that the mothers excreted between 17 and 44% of their 2,3,7,8-TCDD burden by lactation. Based on measurements of 2,3,7,8-TCDD in fat at 4, 12, and 24 months of age, it was found that in the young monkeys, the decline in 2,3,7,8-TCDD in fat followed first-order, single-compartment kinetics, with a half-life of approximately 181 days (Bowman et al. 1990). For the purpose of comparison, the mean half-life in seven adult female Rhesus monkeys was 391 days with standard error of 88 days (Bowman et al. 1989b).

In summary, CDDs can be transferred to the fetus across the placenta and, although the amounts may be relatively small, the transfer may have great biological significance if it occurs during critical periods of organogenesis. Due to their lipophilicity, CDDs can concentrate in human milk and can be transferred to infants through nursing. In general, the amount of individual congeners in human milk decreases as chlorination decreases. Excretion via milk is highest during the first weeks after delivery. Also, the concentration of CDDs in milk is higher in mothers breastfeeding their first child than in those breastfeeding their second child. CDDs transferred to infants through nursing are readily absorbed by the infants. A pharmacokinetic model predicted that the increased body burden in infants that results from breastfeeding does not translate into raised lifetime body burden. Studies in animals have also shown transfer of CDDs across the placenta and via mother's milk.

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

Models are simplified representations of a system with the intent of reproducing or simulating its structure, function, and behavior. PBPK models are firmly grounded in principles of biology and biochemistry. They use mathematical descriptions of the processes determining uptake and disposition of chemical substances as a function of their physicochemical, biochemical, and physiological characteristics (Andersen and Krishnan 1994; Clewell 1995; Mumtaz et al. 2012a; Sweeney and Gearhart 2020). PBPK models have been developed for both organic and inorganic pollutants (Ruiz et al. 2011) and 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 (Mumtaz et al. 2012b; Ruiz et al. 2011; Sweeney and Gearhart 2020; Tan et al. 2020). PBPK models can also be used to more accurately extrapolate from animal to human, high dose to low dose, route to route, and various exposure scenarios and to study pollutant mixtures (El-Masri et al. 2004). 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 (Clewell 1995).

PBPK models for 2,3,7,8-TCDD are discussed below. The pharmacokinetic behavior of 2,3,7,8-TCDD, especially distribution, has been shown to be dose-dependent and involves protein binding and enzyme induction in hepatic tissue. Thus, terms describing these interactions have been included in the animal models described below. Furthermore, since induction of these dioxin-binding proteins is a process mediated by the interaction of a dioxin-receptor (the AhR) complex with specific binding sites on DNA,

additional terms were included in the models. For a detailed explanation regarding the AhR and its involvement in the mechanism of action of 2,3,7,8-TCDD and structurally related halogenated aromatic hydrocarbons, see Section 2.21.

Summary of PBPK Models. Numerous models of 2,3,7,8-TCDD kinetics have been developed that include humans, mice, rats, and pigs. Models have been developed to simulate maternal-fetal and lactational transfer kinetics. Several of these models simulate AhR-mediated induction of CYP1A2 and protein binding of 2,3,7,8-TCDD, which provide more realistic predictions of the effects of these processing on 2,3,7,8-TCDD distribution and elimination. PBPK models of 2,3,7,8-TCDD have been applied in various ways to support risk assessment, including dosimetry extrapolation in derivation of toxicity values, dose reconstruction of past exposures, and prediction of elimination half-lives.

The Kissel and Robarge Model

Description of the Model. The elimination of 2,3,7,8-TCDD from humans was described with a fugacity-based model using physiologically based parameters (Kissel and Robarge 1988). In this model, transport of 2,3,7,8-TCDD was assumed to be perfusion-limited (flow-limited), 2,3,7,8-TCDD was assumed to be uniformly distributed within each tissue group or fluid phase, and tissue levels were considered to be in equilibrium with exiting fluids (blood, urine, bile). Because 2,3,7,8-TCDD appears to be poorly metabolized in humans, the model did not include terms for metabolites. Transport between gut lumen and gut tissue was described as a diffusive process. Included in the differential equations used to solve the system were data for several diets. Body compartment sizes and densities used in the simulations of background exposure and of elimination from individuals with body burdens similar to those of Ranch Hand veterans were based on reference-man data. Tissue perfusion rates and partition coefficients were obtained from the literature. The diet used in all simulations was adapted from the literature and also included a typical intake of added fats and oils. The fugacity capacity of the various diet components, gastric secretions, and fecal materials were either calculated or obtained from the literature. The model was used to predict tissue levels resulting from background exposures, elimination of 2,3,7,8-TCDD from Ranch Hand veterans, and elimination of 2,3,7,8-TCDD from a volunteer.

Validation and Discussion. The steady-state adipose tissue concentrations predicted by the model, assuming no metabolism and a daily background exposure of 50 pg/day in North America, was 7.7 ppt. This value was similar to the lipid-based blood tissue levels reported in the general population with no known unusual exposure. The body burden projected for an intake of 100 pg/day fell outside the typical

range associated with background sources. In simulating the elimination of 2,3,7,8-TCDD from Ranch Hand veterans, the model assumed a background exposure of 50 pg/day and no metabolism. Under these conditions, apparent half-lives of 4.4, 5.2, 5.9, 7.2, 9.1, and 20 years were estimated for individuals with 2,3,7,8-TCDD adipose tissue concentrations of 100, 50, 30, 20, 15, and 10 ppt, respectively. This was in good agreement with a half-life of 7.1 years determined by analysis of blood lipids of veterans with adipose burdens >10 ppt (Pirkle et al. 1989). The results showed that the apparent half-lives increased greatly as tissue concentrations approached the steady-state level associated with background exposure. The model also approximated the uptake efficiency and elimination of 2,3,7,8-TCDD from a volunteer as reported by Poiger and Schlatter (1986). The fact that the predicted uptake efficiency was similar to that found experimentally indicated that the estimated gut-lumen/gut-tissue mass transfer coefficient used was in the appropriate range. The reported half-life was 5.8 years and the model estimated a value of 6.7 years. Overall, the result suggested that a fugacity-based model can provide a viable method for describing overall elimination of 2,3,7,8-TCDD from humans, but it does not provide much insight regarding why elimination occurs in a particular manner.

The Leung et al. Model in Mice

Description of the Model. The model described by Leung et al. (1988) in mice provides quantitative descriptions of the time-course of elimination and levels of 2,3,7,8-TCDD in various organs of C57BL/6J mice and DBA/2J mice, a less-responsive strain with higher body fat content. The model contains five compartments: blood, liver, fat, richly perfused tissues, and slowly perfused tissues. To account for the 2,3,7,8-TCDD binding to receptors in the liver, the model contained two hepatic binding sites: one corresponding to the high-affinity/low-capacity cytosolic AhR and the other to the inducible, low-affinity/high-capacity microsomal protein (CYP1A2). To simulate the intraperitoneal dose route used by Gasiewicz et al. (1983), 2,3,7,8-TCDD was assumed to be absorbed into the liver compartment by a first-order uptake process. Bioavailability was assumed to be 100%. Partition coefficients, physiological parameters, and biochemical constants were obtained or calculated from the literature for each mouse strain. The kidney was assumed to be representative of the richly perfused tissue, whereas the slowly perfused tissue consisted mainly of muscle and skin. The binding capacity of the Ah-less responsive DBA/2J mice was set to equal that of the Ah-responsive mice even though the binding affinity is extremely low. Blood binding was described as a linear process with an effective equilibrium between bound and free 2,3,7,8-TCDD given by a constant. In blood, only one form of 2,3,7,8-TCDD is exchangeable in the tissues, which gives rise to kinetic behavior observed for diffusion-limited uptake into tissues.

Validation and Discussion. The simulation of the time-course of 2,3,7,8-TCDD concentration in the liver and fat of C57BL/6J mice after a single 10 μg/kg intraperitoneal injection generated by the model was in good agreement with the empirical data of Gasiewicz et al. (1983). In trying to simulate the 3-times-higher liver/fat ratio of 2,3,7,8-TCDD in the C57BL/6L mice than in the DBA/2J mice, Leung et al. (1988) varied the fat content parameter in the C57BL/6J mice from 3 to 12% of body weight. The rationale was that the difference in hepatic concentration may have been due to greater capacity of the DBA/2J mouse to sequester the highly lipophilic 2,3,7,8-TCDD in adipose tissue. However, the results showed that the 2,3,7,8-TCDD concentration in the liver was relatively insensitive to body fat content, indicating that this was not an important factor influencing the disposition of 2,3,7,8-TCDD in the liver between the two strains of mice. The study authors also found that the distribution of 2,3,7,8-TCDD was strongly influenced by the binding characteristics of the microsomal binding protein, especially the binding constant. The model gave good simulations of 2,3,7,8-TCDD excretion in both strains of mice. The simulation of the time-course of 2,3,7,8-TCDD concentration in the liver and fat of DBA/2J mice after a single 10 μg/kg intraperitoneal injection was not as good as that for the C57BL/6J mouse if the input was set to be consistent with the uptake and elimination. As with the C57BL/6J mouse, disposition of 2,3,7,8-TCDD in the liver of DBA/2J mice was greatly influenced by the microsomal protein binding constant and rather insensitive to changes in body fat content. The best fit of the empirical data was obtained with a binding constant of 75 nM (20 nM for the C57BL/6J mice), indicating that the 2,3,7,8-binding affinity to the hepatic microsomal protein in the DBA/2J mice was at least 3.5 times lower than that of the C57BL/6J mice.

The Leung et al. Model in Rats

Description of the Model. This model in the Sprague-Dawley rat (Leung et al. 1990b) is an extension of the mouse model previously described and contains the same five compartments and two types of binding proteins: one corresponding to the high-affinity, low-capacity cytosolic 2,3,7,8-TCDD (Ah) receptor, and the other to the inducible, lower-affinity, high-capacity microsomal protein (CYP1A2). In the rat model, both types of binding proteins are defined with their own binding capacities and dissociation constants. The model was used to analyze experimental data for the single-dose studies of McConnell et al. (1984) and Rose et al. (1976), the 7-week Rose et al. (1976) study, the 13-week multiple-dose study of Kociba et al. (1976), and the 2-year feeding study of Kociba et al. (1978). In simulating the single-dose gavage study, 2,3,7,8-TCDD was assumed to be absorbed from the gastrointestinal tract by a first-order uptake process with a rate constant of 0.2/hour. In simulating the multidosing studies, bioavailability was

assumed to be 100%. Physiological parameters, partition coefficients, and biochemical constants were calculated or obtained from the literature. Since there was no literature value for the binding capacity of the microsomal 2,3,7,8-TCDD-binding site in the rat, the value used was approximated by assuming it to be 10 times that of the mouse. The total microsomal binding capacity was apportioned between a basal level and an induced level. Also, AHH activity was taken to be the sum of a basal and induced level. A first-order metabolic rate constant for 2,3,7,8-TCDD metabolism in the liver was adjusted to provide a biological half-life of about 25–30 days.

Validation and Discussion. When the simulation of the McConnell et al. (1984) data for AHH induction included a term for induction of microsomal binding protein, there was good agreement between the simulation and the empirical data. This had not been the case in an initial fitting, which included a constant concentration of microsomal binding protein. Rose et al. (1976) examined the accumulation of 2,3,7,8-TCDD in adipose and liver tissues in rats administered 0.01, 0.1, and 1 μg 2,3,7,8-TCDD/kg/day 5 days/week for 7 weeks; sampling was done at weeks 1, 3, and 7. Model predictions of 2,3,7,8-TCDD concentrations were in good agreement with the experimental data except for concentration in fat at the 0.01 μg/kg/day dose level, in which case the model overpredicted the tissue concentration. Model formulations that had constant microsomal binding capacity overpredicted liver 2,3,7,8-TCDD concentrations at the lower-dose rates. Also, model formulations that contained final amounts of microsomal binding protein (CYP1A2) very different (much higher or lower) from the basal 200 nmol/liver could not simulate 2,3,7,8-TCDD concentration in liver at the highest-dose rate. Similar to the findings in mice, the liver/fat concentration ratio in rats was extremely sensitive to the dissociation constant of the microsomal binding protein. The model simulated well the data from the 7- and 13-week studies (Kociba et al. 1976; Rose et al. 1976), but not as well for data from the 2-year feeding study (Kociba et al. 1978). There was underprediction of 2,3,7,8-TCDD concentration in fat and liver at the low-dose level (0.001 μg/kg/day) and overprediction of the liver concentration at the high-dose level (0.1 μg/kg/day). However, the ratios of the concentrations were consistent with those observed experimentally (1/1 at low doses, much higher in liver at high doses). According to Leung et al. (1990b), the underprediction at the low dose may reflect the fact that the low-dose fat concentration in the 2-year study was close to the limit of detection and thus, subject to more error. At the high dose, physiological parameters such as tissue volume, metabolic constants, and amounts of binding proteins may have been altered by weight loss and changes in body composition, known effects of chronic-duration exposure to 2,3,7,8-TCDD. Leung et al. (1990b) indicated that the overprediction at the high dose could have been due to a loss of microsomal 2,3,7,8-TCDD-binding sites in the chronically exposed rats. The affinity of 2,3,7,8-TCDD for the microsomal binding protein appeared to be greater in the Sprague-Dawley rats than

in C57BL/6J mice, which could account for the higher liver/fat concentration ratio in rats than in mice, assuming that the partitioning between tissues is approximately the same in the two species.

Wang et al. (1997) extended the work of Leung et al. (1988, 1990b) and Andersen et al. (1993) and developed an improved model to describe the disposition of 2,3,7,8-TCDD in multiple tissues from female Sprague-Dawley rats. The model of Wang et al. (1997) improved previous modeling attempts in some specific areas such as: (1) providing information on distribution of 2,3,7,8-TCDD at early time points (important for determining unique parameters related to mass transfer such as permeability); (2) better handling of mass balance when considering 2,3,7,8-TCDD binding to plasma proteins; and (3) improved estimation of physical and biochemical parameters. The Wang et al. (1997) model accurately described the time course distribution of 2,3,7,8-TCDD following a single oral dose, as well as the concentration of 2,3,7,8-TCDD in eight target tissues on day 3 after six different doses. The model described by Wang et al. (1997) was coupled to a biologically based pharmacodynamic (BBPD) model to quantitatively describe the relationship between disposition and response in multiple tissues (Santostefano et al. 1998). This later model incorporated both pharmacokinetic and pharmacodynamic events to account for the ability of 2,3,7,8-TCDD to induce CYP1A1 and the fact that CYP1A2 is responsible for maintaining high concentrations of 2,3,7,8-TCDD in the liver. The results showed that the BBPD model accurately described the time course of CYP1A1 protein expression and EROD activity in the liver, skin, and kidneys. It also confirmed that EROD activity can be an appropriate marker for CYP1A1 protein expression, and the shape of the induction curves supported the hypothesis that similar time-dependent mechanism of 2,3,7,8-TCDD-induced CYP1A1 protein expression and associated EROD activity occurs in multiple tissues. This, in turn, suggested that parameter estimation in the study accurately described the AhR-mediated mechanism on protein expression and enzymatic activities in multiple tissues.

Emond et al. Model

Description of the Model. Emond et al. (2004), simplifying the Wang et al. (1997) model, developed a model with four compartments (liver, fat, placenta, and rest of the body) for the dam and one compartment for the fetus. The maternal compartments were described as diffusion limited and the model assumes simple diffusion of 2,3,7,8-TCDD between the placental and fetal compartments (no blood flow to the fetal compartment is assumed). Additionally, the model includes 2,3,7,8-TCDD induction of CYP1A2 and binding in the liver. All parameters for the nonpregnant animal (only fat, liver, and rest of the body compartments were activated) were adapted from Wang et al. (1997) and parameters for growth

of the placental, blood, and fetal compartments and blood flow rates were based on data from O'Flaherty (1994) and Buelke-Sam et al. (1982a, 1982b).

Validation and Discussion. The Emond et al. (2004) model was validated by comparisons of simulations to the Wang et al. (1997) model and using experimental data for four scenarios: acute-duration 2,3,7,8-TCDD exposure in nonpregnant rats, intermediate-duration 2,3,7,8-TCDD exposure in nonpregnant rats, single exposure to 2,3,7,8-TCDD on GD 15, and intermediate-duration 2,3,7,8-TCDD exposure prior to mating and continuing throughout gestation. Reasonable agreements were found in these comparisons (typically within 20–30% of the experimental data). The investigators noted that limited data are available to develop and validate the developmental model and that extrapolation to humans should be done with caution. The model was subsequently modified to include inducible hepatic elimination, which describes the elimination rate as a function of CYP1A2 induction (Emond et al. 2006). The Emond et al. (2004) model has also been extrapolated to humans (Emond et al. 2005). This model was optimized using serum 2,3,7,8-TCDD levels from 20 Ranch Hand veterans and data from a subject ingesting a single dose of 2,3,7,8-TCDD and followed for 40 days, and the model was validated using an additional 10 Ranch Hand veterans and data from 2 Austrian women. A good correlation between predicted blood concentrations and measured blood concentrations was found for both groups of subjects. EPA (2012a) reported parameter values for human, rats, and mice and used the models for dosimetry extrapolation in deriving toxicity values for 2,3,7,8-TCDD (EPA 2012a).

Emond et al. (2018) applied the mouse model (EPA 2012a) to simulate effects of obesity on 2,3,7,8-TCDD kinetics. Feeding a high fat diet to C57BL/6J mice resulted in obesity and an increase in the terminal blood elimination half-life for radiolabeled 2,3,7,8-TCDD following a single oral dose of [^{3}H]-labeled 2,3,7,8-TCDD. The PBPK model did not predict the increase in half-life even when the model was adjusted to account for the increase in body mass and fat content of the mice, suggesting that other factors were responsible for the increase in the half-life.

Emond et al. Maternal Model

Description of the Model. The Emond et al. (2005, 2006) human model was expanded to simulate transplacental transfer of 2,3,7,8-TCDD to the developing fetus and transfer from human milk to the nursing child (Emond et al. 2016, 2017). Parameter values and sources for the values are reported in Table 1 of Emond et al. (2016). The maternal-fetal model includes compartments representing the mammary gland, placenta, and fetus. Transfer of 2,3,7,8-TCDD from blood to placenta is simulated as a

diffusion-limited process governed by a placental diffusion permeability coefficient. Exchanges between the placenta and fetus are simulated as diffusion-limited processes governed by the concentrations in the placenta and fetus and a clearance coefficient (mg/hour). Maternal-fetal transfer is assumed to begin at 8 weeks of pregnancy. Transfer of 2,3,7,8-TCDD to the nursing infant is simulated as a balance between transfer to human milk from maternal blood and transfer out of milk from nursing. The model simulates flow-limited transfer between maternal blood and mammary tissue blood and first order (day^{-1}) transfer from mammary blood to milk. Kinetics parameters governing lactational transfers were optimized to achieve a maternal blood/milk concentration ratio reported by Schecter et al. (1995, 1996a). Transfers to the nursing infant assumed 150 mL of human milk consumed in each feeding. The model is configured to be able to simulate multiple pregnancies. A sensitivity analysis of the Emond et al. (2016) model showed that the predicted area under the curve for maternal blood levels was most sensitive to parameters governing the induction of CYP1A2, absorption from the gastrointestinal tract, and the adipose/blood partition coefficient.

Validation and Discussion. Emond et al. (2016) applied the model to simulate that time profile for blood 2,3,7,8-TCDD in two cohorts of women exposed during the Seveso accident. Women in cohort A were 4–39 years of age at the time of the accident (n=23). Women in cohort B were 3–17 years of age at the time of the accident (n=18). Oral exposure was adjusted to achieve the best fit to the observed blood levels in individual women (all exposure was attributed to oral). A linear model applied to the observed and predicted maternal blood concentrations showed that the PBPK model explained approximately 85% of the variance in the observed blood levels of cohort A (r^2 0.8457) and tended to underpredict the observations by approximately 23% (regression slope 0.774 with observed as the independent variable). The PBPK model explained 100% of the observed variance in cohort B (r^2 0.9976, regression slope 0.995).

Emond et al. (2017) applied the model to simulate blood serum 2,3,7,8-TCDD (TEQ) levels reported for various exposure cohorts. Oral exposure was adjusted to achieve the best fit to observed blood levels. The model predicted the age-dependent increase in serum TEQ observed in the Calcasieu Parish Louisiana cohort (Wong et al. 2008; see Figure 1 of Emond et al. 2017). The model also predicted the observed declines in serum 2,3,7,8-TCDD levels in a subset of the Operation Ranch Hand cohort, Seveso women cohort, and a cohort exposed from operations of a hazardous waste incinerator in Spain (Michalek et al. 1996, 1997; Schuhmacher et al. 2014; Wong et al. 2008; see Figures 4, 5 and 6 of Emond et al. 2016).

Joffin et al. Model

Description of the Model. Joffin et al. (2018) extended the Emond et al. (2004; EPA 2012a) mouse model to include a skin compartment. The model was applied to simulate disposition of 2,3,7,8-TCDD in C57BL/6J mice that received subcutaneous skin xenografts of adipose tissue collected from mice dosed with 2,3,7,8-TCDD. The model simulated first order ($hour^{-1}$) release of 2,3,7,8-TCDD from the adipose graft to the skin compartment. Transfer of 2,3,7,8-TCDD from skin to blood was simulated as a diffusion-limited process governed by a skin permeability coefficient. Blood flow to the graft (from skin) was assumed to begin 4 weeks after the graft. Parameter values and bases for the values are reported in Table 1 of Joffin et al. (2018). Values for the first-order rate coefficient for transfer of 2,3,7,8-TCDD from graft to skin, skin permeability coefficient, and graft permeability fraction were optimized against observations of graft 2,3,7,8-TCDD concentrations in preliminary experiments.

Validation and Discussion. The model predicted the observed liver and adipose concentrations in donor mice that received an intraperitoneal dose of 2,3,7,8-TCDD (10 μg/kg). The model also simulated the time course for the decrease in 2,3,7,8-TCDD concentration in the graft and buildup of concentration in the host adipose tissue and liver.

The Andersen et al. Model

Description of the Model. This model (Andersen et al. 1993) is an extension of the earlier PBPK models developed by Leung et al. (1988, 1990b) for 2,3,7,8-TCDD. Like the earlier models, this model consists of five compartments. Each of the four tissue compartments has a specified blood flow, tissue compartment volume, and tissue blood volume. Movement of chemical from blood to tissue was modeled to be proportional to the product of a permeation coefficient times surface area for the tissue. When this product is lower that the specified blood flow for the tissue, tissue uptake is diffusion-limited. Because of the diffusion-limited tissue compartments, the model did not require blood binding to match the time-course of tissue uptake. It was assumed that in the liver both the AhR and the inducible binding protein act to sequester 2,3,7,8-TCDD through a capacity-limited binding process, and the binding protein was assumed to be CYP1A2. Binding interactions with CYP1A2 and CYP1A1 were described by reversible equilibrium relationships, which is valid as long as the rate constants for association/dissociation are large. It was also assumed that the DNA sites to which the Ah-2,3,7,8-TCDD complex binds are present at much lower concentrations than the Ah-ligand complex. For both CYP1A1 and CYP1A2 induction, it was assumed that the Ah-ligand complex formation was equivalent, but that

the Hill term, *n* (a measure of interaction for multiple Ah-ligand complex binding sites), and the Hill binding constant were different for the two responses. The model also allowed for autoinduction of metabolism following 2,3,7,8-TCDD treatment. Data from Abraham et al. (1988) and Krowke et al. (1989) were analyzed. The former study provided dose-response characterization of concentrations of 2,3,7,8-TCDD in liver and of liver CYP1A1 activity and time-course characterization of 2,3,7,8-TCDD concentration in tissues and enzyme activities in female Wistar rats. Krowke et al. (1989) examined liver and fat concentrations in male Wistar rats dosed weekly for up to 6 months. In addition, Andersen et al. (1993) examined the potential correlation between several measures of dose estimated by the model and the promotional efficacy and carcinogenicity of 2,3,7,8-TCDD in Sprague-Dawley rats. Cancer data from Kociba et al. (1978) and Pitot et al. (1980) were analyzed.

Validation and Discussion. Abraham et al. (1988) found that the disposition of 2,3,7,8-TCDD in liver and fat from rats administered a single subcutaneous dose (0.001–10 μg/kg) of the chemical was highly dose-dependent. The disproportionately higher concentration in the liver at higher doses appeared to be due to induction of a dioxin-binding protein, presumably CYP1A2. The model developed by Andersen et al. (1993) successfully simulated the experimental data. The affinity of the binding protein was estimated to be 6.5 nmol, while a value of 1 for *n* suggested little interaction among 2,3,7,8-TCDD-responsive DNA-binding sites involved in the expression of CYP1A2. For describing induction of CYP1A1, an *n* of 2.3 was required, which suggested possible interactions among DNA-binding sites for the Ah-ligand complex with this gene. The simulation of the time-course of elimination from liver and of induction of CYP1A1 was in good agreement with the empirical data but required the inclusion of time-dependent growth parameters over the 100 days of the experiment. The model also successfully simulated the data from the repeated-dosing study by Krowke et al. (1989) after small adjustments were made to fat and slowly perfused tissue parameters. The measures of dose that were used for comparison with the promotional and carcinogenic properties of 2,3,7,8-TCDD were integrated total liver concentration during the treatment period, or integrated free liver 2,3,7,8-TCDD concentration. Also, measures of tissue dose related to enhanced expression of CYP1A1 and hepatic binding proteins were calculated and examined for correlation with promotional activity. Results of the analysis revealed that under the exposure conditions, the tumor promotional response of 2,3,7,8-TCDD in the rat liver most closely correlated with integrated expression of the CYP1A1 gene. However, Andersen et al. (1993) indicated that since there is no expectation of causality between tumor responses and induction of CYP1A1 (or CYP1A2), the correlation should be regarded cautiously. Consistent with the findings of Leung et al. (1988, 1990b), the results from the Andersen et al. (1993) study showed that over a certain dose range (e.g., at doses several fold above background), protein (CYP1A2) induction greatly alters 2,3,7,8-TCDD disposition.

Andersen and co-workers developed a model of hepatic enzyme zonation that was combined with the PBPK model of protein induction (Andersen et al. 1993) to create a multicompartmental representation of the liver architecture that can be used to predict the degree of induction in both the whole liver and in specific regions (Andersen et al. 1997a, 1997b). A geometric representation was used to divide functional units (based on enzyme distribution) within the liver into five zones. The primary objective was to compare model predictions for regional induction with regional protein induction as visualized by immuno-histochemistry. The data set modeled included analysis of tissue distribution of 2,3,7,8-TCDD in the first days or weeks after a single dose, time course studies for about 100 days after a single dose, and initiation-promotion studies in rats dosed for up to 6 months. The results showed that the five-compartment model was more successful than conventional homogeneous one-compartment liver models not only in simulating low-dose behavior for mRNA in whole liver, but also in representing immunohistochemical observations. Five or more compartments were required to give a sharp boundary between induced and noninduced regions of the liver. When the five-compartment liver model was used to account for CYP1A1 and CYP1A2 induction and regional distribution of induced enzymes, the low-dose behavior appeared to be nonlinear and was better described, with a large *n* value (Hill coefficient) and a range of affinities in the liver covering about 81-fold differences between centrilobular and periportal regions.

The Kohn et al. (National Institute of Environmental Health Sciences [NIEHS]) Model

Description of the Model. Kohn et al. (1993) constructed a mathematical model (the NIEHS model) to describe 2,3,7,8-TCDD tissue distribution and 2,3,7,8-TCDD-mediated alterations in hepatic proteins in the rat. The model assumed that 2,3,7,8-TCDD mediates increases in liver concentration of transforming growth factor-α (TGF-α) by a mechanism which requires the AhR. TGF-α subsequently binds to the EGF receptor, a process that is known to cause internalization of the receptor in hepatocytes. This is thought to be an early event in the generation of a mitogenic signal. The model included equations for the AhR-dependent induction of CYP1A1 and CYP1A2 activity and of the AhR itself. Because it was also assumed that estrogen action is required for 2,3,7,8-TCDD-mediated induction of TGF-α, production of the estrogen receptor, CYP1A2-catalyzed formation of catechol estrogens, and deactivation of estrogens by glucuronidation were included in the model. The model predictions were compared to the two-stage initiation-promotion data of Tritscher et al. (1992) and Sewall et al. (1993). Gavage doses equivalent to 3.5–125 ng 2,3,7,8-TCDD/kg/day for 30 weeks were used in these studies. Data from Abraham et al. (1988) were also analyzed. Model parameters were obtained from the literature or calculated from

experimental data and adjusted to make the model reproduce the observations of Tritscher et al. (1992) and Sewall et al. (1993).

Validation and Discussion. The model prediction for the percentage of absorption (>90%) from ingestion of 2,3,7,8-TCDD was in good agreement with experimental data of Rose et al. (1976). The model also predicted that 92.2% of the metabolite appears in the feces and 7.8% in the urine at a dose of 125 ng/kg/day. The dose of 2,3,7,8-TCDD did not have a significant effect on these predictions. From the fit to the data of Abraham et al. (1988), the model predicted an initial and overall half-time clearance from liver of 11.8 and 13.5 days, respectively, which is very close to the experimentally obtained 11.5 and 13.6 days. Similar good agreement was obtained for half-time elimination from fat (22.3 days versus 24.5 days). The model predicted a linear relationship between administered dose and the concentration of 2,3,7,8-TCDD in the liver at doses between 3.5 and 125 ng/kg/day, which was in good agreement with the data of Tritscher et al. (1992). The relationship between 2,3,7,8-TCDD dose and induction of both CYP1A1 and CY1A2 was best fit by a hyperbolic curve suggesting lack of cooperative interactions among binding sites. The hyperbolic curve was consistent with the experimental data for induction of these proteins from Tritscher et al. (1992). The model also predicted that the fractional occupancy of the AhR by 2,3,7,8-TCDD rises from 13.4% at a dose of 3.5 ng/kg/day to 69.3% at 125 ng/kg/day. The model prediction of the degree of internalization of the EGF receptor as a function of the concentration of TGF-α was also hyperbolic in shape and successfully reproduced the experimental data of Sewall et al. (1993). Kohn et al. (1993) indicated that as this response may be involved in the mechanism of tumorigenesis in 2,3,7,8-TCDD-treated rats, it would be expected that it would correlate with tumor incidence better than does tissue dose. If so, extrapolation of effects at high dose to low doses may underestimate low-dose effects. However, extrapolation from low dose to extremely low dose would still be valid. The model predicted that 10 days after administration of a single dose of 1 μg 2,3,7,8-TCDD/kg, there should be a greater decrease in plasma membrane EGF receptor in female rat liver than in male rat liver, which is consistent with the observed lower sensitivity of the male. Consistent with the experimental data, the model reproduced the decrease in hepatic estrogen receptor (ER) level resulting from exposure to 2,3,7,8-TCDD, and the relationship between concentration of 2,3,7,8-TCDD and amount of receptor was also hyperbolic. Overall, the model's success in reproducing the observed responses to 2,3,7,8-TCDD for the various proteins included in the model supports the proposed mechanism that internalization of the EGF receptor in response to induction of TGF-α may be the origin of the mitogenic signal important for carcinogenesis.

Kohn et al. (2001) updated the Kohn rat PBPK model (described in ATSDR 1998) to include multiple TCDD-liganded AhR binding sites for CYP1A1 and CYP1B1 genes, a lag of 0.2 days for production of mRNA and induced proteins, and stabilization of mRNA by a poly(A) tail. The model was validated using observed liver TCDD levels from the literature. In general, there was good agreement between the predicted and measured tissue TCDD concentrations and the dose-response curves for CYP1A1, CYP1A2, and CYP1B1.

The Carrier et al. Model

Description of the Model. The first part of this model provides a quantitative description of the distribution of 2,3,7,8-substituted CDDs (and CDD-like compounds) between liver and adipose tissues as a function of overall body concentration at any given time (Carrier et al. 1995a). In a second step, differential equations were used to describe the disposition of CDDs in liver, adipose tissues, and whole body as a function of time (Carrier et al. 1995b). The first step of the model was based on several hypotheses: (1) changes in overall CDD concentration are slow relative to inter-tissue diffusion exchanges, protein induction, and binding of CDDs in the liver; (2) CDDs are mainly in adipose tissue and in the liver, but exchanges between these two sites are mediated via the blood; (3) the liver synthesizes proteins that bind free CDDs according to standard mass action association-dissociation mechanisms; (4) synthesis of binding proteins in the liver is linked to binding of free CDDs to the AhR; (5) CDDs in fat deposits within the liver contribute to the overall liver burden and is taken into account; and (6) small amounts of CDDs are contained in organs other than the liver and adipose tissues, and this fraction is assumed to be constant. In the second step, CDDs were assumed to be eliminated mainly by hepatic clearance; elimination by lactation or transplacental distribution was not considered. Model simulations of various experimental data sets, as specified below, were conducted. When not readily available, anatomical and physicochemical parameters were obtained from laboratory or clinical data.

Validation and Discussion. The model successfully simulated data from Abraham et al. (1988), who provided dose-response characterization of concentrations of 2,3,7,8-TCDD in the liver of rats after a single dose of the compound. Analysis of the data showed that the higher the body burden, the higher the proportion of the burden contained in the liver. However, the model predicted that a plateau is reached when body burden is >1 mg 2,3,7,8-TCDD/kg body weight. The model predictions were also in good agreement with experimental data from Van den Berg et al. (1986a), who administered a single dose of a mixture of CDDs and CDFs to rats and hamsters and with data in monkeys administered a single oral dose of 2,3,7,8-TCDD (McNulty et al. 1982). Results from simulations conducted on data from chronic-

duration studies in rats (Kociba et al. 1978; Rose et al. 1976) and on human data from Yusho patients also showed that increasing the body burden results in an increase in the fraction of the body burden present in the liver and in an increase in the liver/adipose concentration ratio. These changes in fractional distributions were attributed to the affinity of specific liver proteins for binding of free hepatic CDDs and the saturable capacity of the binding proteins at high concentration of free CDDs. Model simulations of elimination data in rats after single (Abraham et al. 1988) or repeated doses (Kociba et al. 1978; Rose et al. 1976) of 2,3,7,8-TCDD, as well as data from a Yu-Cheng patient agreed well with the empirical data and showed that disposition kinetics of 2,3,7,8-substituted CDDs are nonlinear (i.e., as body burden decreases with time, liver and adipose tissue half-lives increase). According to the model, an additional factor that can influence the disposition kinetics of 2,3,7,8-CDDs is a rapid change in body weight and/or adipose tissue mass. A rapid loss of adipose tissue whether by dieting or in patients experiencing anorexia, would result in a higher concentration of the chemical in the remaining adipose tissue, particularly if the loss of tissue is much faster than whole body elimination via the liver.

Aylward et al. (2005) modified the concentration- and age-dependent model of elimination developed by Carrier et al. (1995a, 1995b) to include the amount of 2,3,7,8-TCDD eliminated through partitioning from circulating lipids across the lumen of the large intestine into the fecal content. The modified model was fit to serial serum 2,3,7,8-TCDD sampling data from two Austrian subjects with a mean follow-up of 2.7–3 years and 36 subjects (19 males and 17 females) from Seveso with a mean follow-up of 16 years. The modified model allows for a better prediction of peak historical exposures using current serum 2,3,7,8-TCDD levels. Aylward et al. (2005) compared the estimated peak serum 2,3,7,8-TCDD levels for the NIOSH cohort back-extrapolated assuming first-order kinetics with a fixed half-life of 7–9 years to peak levels predicted by the modified model and found that assuming first-order kinetics resulted in an underestimation of maximum concentrations by several fold to an order of magnitude.

Maruyama et al. Model

Description of the Model. The Maruyama et al. (2002) PBPK model, which is a modification of the Lawrence and Gobas (1997) fugacity model, consists of six compartments: blood, liver, kidney, fat, muscle, and richly perfused tissue (brain, lung, and spleen). Exposure was assumed to be through diet only. Tissue:blood partition coefficients were determined using autopsy data from eight Japanese men and women exposed to background levels of CDDs and CDFs. For most congeners, the measured values were within the simulated ranges in liver, kidney, and blood; the model underestimated 1,2,3,6,7,8-HxCDD concentrations in the kidney, fat, blood, and muscle.

Validation and Discussion. Predicted mean concentrations of CDD and CDF congeners were compared to measured values from autopsy data for 30 different Japanese persons. Overall, there was good agreement between the estimated and measured values, although the model tended to underestimate CDD levels and overestimate CDF levels. Maruyama et al. (2001) also developed a PBPK model that would allow for the estimation of dioxin concentrations in Japanese breastfed infants. This model was composed of six compartments (liver, kidney, fat, blood, muscle, and richly perfused tissues) and the source of exposure was presumed to be human milk exclusively.

Savvateeva et al. Model of Pigs

Description of the Model. Savvateeva et al. (2020) developed a model to simulate the kinetics of ingested 2,3,7,8-TCDD in growing pigs. The model includes three compartments representing the blood, adipose, and liver. The adipose and liver compartments are subdivided into tissue and extracellular compartments. Ingested 2,3,7,8-TCDD enters the blood compartment with the rate governed by a dose-dependent absorption fraction and is distributed to the extracellular compartments of adipose and liver. Transfer to adipose and liver tissue is assumed to be diffusion-limited governed by permeability coefficients. Elimination is simulated as first-order excretion from blood and inducible first-order metabolism in liver. Induction of metabolism (CYP1A2) is simulated as an AhR-mediated response (Emond et al. 2005; Wang et al. 1997). Growth of the body and adipose weights were simulated with a logistic equation fit to observations on pig growth (Savvateeva et al. 2020). Blood and liver weights were assigned values of 6 and 3% of body weight, respectively (Savvateeva et al. 2020). Partition coefficients were predicted based on physical-chemical properties and predicted values compared to measured tissue and blood concentrations observed in pigs dosed with 2,3,7,8-TCDD.

All other chemical parameters were optimized to observations made on pigs following 13 weeks of daily oral dosing with capsules containing a mixture of CDD and CDF congeners. The daily doses increased with body weight. Ranges for the three dose groups were: low, 0.128–0.364 ng; middle, 1.874–5.353 ng; and high, 17.005–48.584 ng. At 13 weeks, the per kg doses for 70 kg pigs were approximately 0.005, 0.076, and 0.69 ng/kg/day, for the low-, middle-, and high-dose groups, respectively. Optimization relied on observations of 2,3,7,8-TCDD concentration in blood, liver, and adipose following exposure in the three dosing groups.

Validation and Discussion. The optimized model predicted the time course for the decline in adipose 2,3,7,8-TCDD concentration following dosing (measured from fat biopsies collected at three time points). The model also predicted blood and liver concentrations measured 60 days following dosing. The model was applied to predicting elimination half-lives of 2,3,7,8-TCDD in blood, adipose, liver, and whole body. These values were 19–25 days in the low-dose group, 19–24 days in the middle-dose group and 14–21 days in the high-dose group. The model was used to predict adipose TEQ concentrations following a period in which pigs had been accidentally exposed to contaminated feed in Belgium (Covaci et al. 2007).

Risk Assessment. In early efforts to model the disposition of persistent PAHs, disposition was described by simple partitioning between the blood and the various tissues with first-order metabolism in the liver. In those studies, the role that extensive tissue binding to particular cellular proteins might play in determining the overall disposition of the chemical was not accounted for. In contrast, the descriptions of Leung et al. (1988, 1990b) and Carrier et al. (1995a, 1995b) attempted to provide a biochemical basis for the observed tissue distribution. The use of this type of model may help explain interspecies differences in 2,3,7,8-TCDD sensitivity and carcinogenicity. The rodent PBPK model for 2,3,7,8-TCDD revealed very consistent behavior between species, and some of the predictions of high dose-low dose behavior were verified.

One advantage of a description that explicitly includes protein binding is the ultimate ability to develop pharmacodynamic models for 2,3,7,8-TCDD (and related chemicals) toxicity based on AhR occupancy or Ah-TCDD complex concentration *in vivo* and to realistically couple it with the biologically based cancer models (or with models for other 2,3,7,8-TCDD responses). This was attempted by Andersen et al. (1993) and Kohn et al. (1993), who included estimates of binding constants between the AhR and 2,3,7,8-TCDD and between the AhR-dioxin complex and sites on DNA. Santostefano et al. (1998) extended previous modeling attempts by determining parameter values based on time course of CYP1A1 and CYP1A2 responses in multiple tissues using a simultaneous PBPK and PBPD models. However, as noted by Andersen et al. (1993), in order to develop a complete biologically motivated risk-assessment model, these dosimetry models need to be combined with quantitative descriptions of cell and tissue responses. Kohn et al. (1993) used the NIEHS model to successfully predict tissue concentrations of 2,3,7,8-TCDD and of various induced proteins involved in the carcinogenic response to 2,3,7,8-TCDD and suggested that such a model might permit extrapolation of responses beyond the range obtained from experimental data and lead to scientifically sound approaches for estimating risks of adverse health effects of exposure to 2,3,7,8-TCDD. The importance of the results of Kohn et al. (1993) can be illustrated by

the finding that the dose-response curves for various proteins were hyperbolic rather than sigmoid. A sigmoid dose-response relationship in the response requires a higher concentration to produce a given response at a low dose than a hyperbolic response having the same concentration for half-maximal effect. This implies that the response is approximately linear at very low doses. Expansion of previous models to include maternal-fetal and maternal-infant transfer of 2,3,7,8-TCDD provided a basis for dosimetry extrapolation to support derivation of toxicity values for developmental endpoints (Emond et al. 2005, 2006; EPA 2012a).

3.1.6 Animal-to-Human Extrapolations

As discussed in Section 2.1, there are a number of limitations in the human database; for most health effects, the data are inadequate to assess the potential for humans having a particular effect. Because the human data are incomplete, hazard and risk must be extrapolated across species. A large number of adverse effects have been observed in animals, and most have been observed in every experimental animal species tested, if the appropriate dose is administered. This is illustrated in Table 3-2 for eight major effects associated with CDD toxicity (acute lethality, hepatotoxicity, wasting syndrome, chloracne, immunotoxicity, reproductive toxicity, developmental toxicity, and cancer). With the exception of acute lethality in humans, positive responses have been observed in each tested species, when a response has been investigated. Despite the similarities in hazard response between different species, large species differences in sensitivity have been observed. Comparisons of species sensitivity demonstrate that no species is consistently sensitive or refractory for all effects and, for some effects, there is a small range of species sensitivity. As presented in Table 3-3, the range of LD_{50} values for six commonly tested animal species spans several orders of magnitude. Guinea pigs have the lowest LD_{50} value (0.6 μg/kg) and hamsters have the largest (1,157 μg/kg). However, if these outliers are removed, the range of LD_{50} values for mice, monkeys, rabbits, and rats is less than an order of magnitude (22–115 μg/kg). The range of LOAELs for developmental effects (increases in mortality and hydronephrosis) were typically within an order of magnitude. In contrast, the range of ED_{50} values for thymic atrophy spans more than 2 orders of magnitude, with guinea pigs (0.8 μg/kg) being the most sensitive and mice the least sensitive (280 μg/kg). These data suggest that even though some effects have wide ranges of sensitivity, for most of the effects, the LOAELs for the majority of species cluster within an order of magnitude (Table 3-3).

Table 3-2. Comparison of Health Effects Among Species Exposed to CDDs

Effect	Human	Monkey	Rat	Mouse	Hamster	Dog	Rabbit	Guinea pig	Mink
Acute lethality	–	+	+	+	+	+	+	+	+
Hepatotoxicity	+	+	+	+	ND	ND	ND	+	+
Wasting syndrome	**	+	+	+	+	ND	ND	+	+
Chloracne	+	+	ND	+	ND	ND	+	ND	ND
Immunotoxicity (thymic atrophy)	ND	+	+	+	+	ND	ND	+	ND
Reproductive toxicity (loss of pregnancy)	**	+	+	+	ND	ND	+	+	ND
Developmental toxicity (fetal toxicity and/or mortality)	**	+	+	+	+	ND	+	+	ND
Cancer	+	ND	+	+	+	ND	ND	ND	ND

+ = observed; – = not observed; ** = some effects have been observed but data limitations preclude drawing conclusions; CDD = chlorinated dibenzo-*p*-dioxin; ND = no data

Table 3-3. Comparison of LOAELs Among Animal Species Following a Single Oral Dose of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

	LOAEL (µg/kg)			
	Death	Immunological effects	Developmental effects	
Species	LD_{50}	Thymic atrophy (ED_{50})	Fetal/pup mortality	Hydronephrosis
Guinea pig	0.6 (Schwetz et al. 1973)	0.8 (Hanberg et al. 1989)	1.5 (Olson and McGarrigle 1992)	ND
Hamster	1,157 (Olson et al. 1980a)	48 (Hanberg et al. 1989)	18 (Olson and McGarrigle 1992)	1.5 (Olson and McGarrigle 1992)
Mouse	100 (Weber et al. 1995)	280 (Hanberg et al. 1989)	10 (Mustafa et al. 2008)	0.5 (Silkworth et al. 1989b)
Monkey	70 (1/3 died) (McConnell et al. 1978a)	ND	1 (McNulty 1984)	ND
Rabbit	115 (Schwetz et al. 1973)	ND	ND	ND

Table 3-3. Comparison of LOAELs Among Animal Species Following a Single Oral Dose of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (2,3,7,8-TCDD)

	LOAEL (µg/kg)			
	Death	Immunological effects	Developmental effects	
Species	LD_{50}	Thymic atrophy (ED_{50})	Fetal/pup mortality	Hydronephrosis
Rat	22 (Schwetz et al. 1973)	26 (Hanberg et al. 1989)	0.7 (Bjerke and Peterson 1994; Bjerke et al. 1994a; Ishimura et al. 2002; Kransler et al. 2009; Tomasini et al. 2012)	1 (Nishimura et al. 2006)

ED_{50} = median effective dose; LD_{50} = median lethal dose; ND = no data

It is generally accepted that the AhR plays a role in mediating many toxic responses attributed to exposure to CDDs (for additional information on the mechanisms of toxicity, see Section 2.21). For some responses, receptor binding appears necessary but may not be sufficient to result in downstream biological effects. AhRs have been found in most species, including humans, monkeys, rats, mice, hamsters, rabbits, and guinea pigs (Denison et al. 1986; Landers and Bunce 1991). A simple way to explain sensitivity differences among species to 2,3,7,8-TCDD and related compounds, at least for AhR-mediated responses, would be to assume that they are related to differences in receptor levels in target tissues and/or to differences in the affinity of binding of the specific CDD congeners. However, experimental data indicate that differences in such parameters cannot explain marked differences in CDD toxicity across species. For example, single-dose LD_{50} values range from 0.6 µg/kg in guinea pigs to 1,157 µg/kg in hamsters, but the affinity with which 2,3,7,8-TCDD binds to the AhR from guinea pigs is not significantly different from the affinity with which 2,3,7,8-TCDD binds to the hamster AhR (Denison et al. 1986). In addition, there are no significant differences in the level of the hepatic AhR between the two species, suggesting that in addition to species differences in receptor levels and in their affinities for the ligand, differences in species sensitivity to 2,3,7,8-TCDD may be determined by some event or events occurring after the initial binding of 2,3,7,8-TCDD to the AhR. These late events may involve a complicated interplay between genetic and environmental factors, which may be key determinants of 2,3,7,8-TCDD biological potency and toxicity. Factors unrelated to the AhR, such as toxicokinetic differences, may also account for some of the observed species differences (for additional information, see Section 2.21). The AhR has been identified in many human tissues and human cell lines (Okey et al. 1994). However, considerable individual differences in the expression levels of both AhR and ARNT mRNAs have been found in human tissues (Hayashi et al. 1994). Furthermore, based on findings in

inbred mice, polymorphism in the AhR probably exists in humans, so that a concentration of TCDD that produces a response in one individual may not do the same in another (Whitlock 1993). This could explain why there was a wide range of serum 2,3,7,8-TCDD levels among Seveso residents where the occurrence of chloracne was sporadic over a generally wide range of doses (Mocarelli et al. 1991).

The weight of evidence from animal species comparisons and mechanistic data indicates that caution should be exercised when extrapolating from animals to humans. Some theoretical models indicate a basis for extrapolating from animals to humans, but such models have not been validated; there is wide variation in the results of different models and a great deal of uncertainty remains regarding whether valid, predictive extrapolations can be made. It is reasonable to assume that humans will not be the most sensitive responder or be refractory to all effects, and that they will have a wider range of response due to increased heterogeneity. Levels of exposure to CDDs that produce toxicity in experimental animals cannot be directly compared to levels associated with adverse health effects in humans because most epidemiologic studies do not provide adequate data to estimate CDD exposures in the studied populations.

Several factors need to be considered when understanding species differences in CDD toxicity, in particular 2,3,7,8-TCDD toxicity. One of the primary factors is binding affinity, and quantitative measures of binding affinity can serve as a preliminary indicator of species susceptibility to 2,3,7,8-TCDD. Events in the AhR signaling pathway, such as binding of cofactors (e.g., ARNT) or chaperone proteins, translocation to the nucleus, and interaction with transcriptional control elements on DNA, and transcriptional cofactors could also affect 2,3,7,8-TCDD responsiveness. However, the limited available data on species differences in these downstream AhR signaling events have not found marked differences, and none of the available data suggest that a species with a low binding affinity would have a high responsiveness to 2,3,7,8-TCDD (Connor and Aylward 2006). The binding affinities (expressed as the dissociation constant, K_d) from several species are presented in Table 3-4 (note that the dissociation constant is inversely proportional to the binding affinity). The dissociation constant in humans is approximately 10-fold higher than most laboratory species, indicating approximately one-tenth of the binding capacity. Molecular genetic studies have compared the human AhR to the AhRs in C57BL/6 mice (2,3,7,8-TCDD-responsive strain) and DBA/2 mice (2,3,7,8-TCDD-nonresponsive strain). Two single nucleotide changes are believed to be responsible for the differences in the binding affinity and function between the AhR in C57BL/6 mice and DBA/2 mice (Connor and Aylward 2006; Harper et al. 2002). In DBA/2 mice, the single nucleotide change in the ligand binding domain of the receptor causes valine to be substituted for alanine, resulting in decreased binding affinity (Harper et al. 2002). The

second nucleotide change (leucine to proline) in the DBA/2 mouse converts a stop codon into an arginine, resulting in a longer transcript and extending the C-terminal portion of the AhR protein (Harper et al. 2002). Humans have both "DBA-type" mutations, whereas Sprague-Dawley rats, Golden Syrian hamsters, and domestic guinea pigs have the leucine and proline mutations (Connor and Aylward 2006).

Table 3-4. AhR-TCDD Equilibrium Dissociation Constants (K_d) in various Laboratory Species and Human Tissues

Species/strain	Dissociation constant[a] (K_d, nM)
Hartley guinea pig	0.06, 0.16
Gerbil	0.20
Syrian hamster	0.22, 0.33
Sprague-Dawley rat	0.12, 0.22, 2.4
C56BL/6 (N or 1) mouse	0.29, 0.27, 0.52, 0.5–1.6, 1.1, 1.8
B6D2F1 mouse	0.42
Long-Evans rat	3.4
Han/Wistar (Kuopio) rat	3.9
Wistar rat	5.4
Cynomolgus monkey	0.26, 16.5
Swine	17.5
Beagle dog	17.1
DBA/2 mouse	16
Human liver	18.6
Human lymphoblastoid cells	4.6
Human tonsils	17.6
Human placenta	9.6

[a]For some species, values were taken from multiple studies.

AhR = aryl hydrocarbon receptor; TCDD = tetrachlorodibenzo-*p*-dioxin

Source: Connor and Aylward (2006)

Using *in vitro* and *in vivo* data, Connor and Aylward (2006) compared the biological responses of humans and laboratory animals to assess whether differences in binding affinity and molecular structure of the AhR translate to differences in biological responsiveness (as assessed by induction of CYP1A1 and CYP1A2). The ratios of human EC_{50} to rat EC_{50} values for EROD activity, measured *in vitro*, were 8–34, suggesting that approximately 10-fold or higher 2,3,7,8-TCDD levels were needed in human cells to elicit the same response as rat cells, which is consistent with the comparison of ligand binding affinities. Comparisons were also made using *in vivo* data by measuring gene expression of *CYP1A1* (as mRNA) in Seveso residents and German herbicide manufacturing workers. No detectable increases in gene

expression of CYP1A1 were found at body burdens of ≤250 ng TEQ/kg; at body burdens of ≥750 ng TEQ/kg, increases in gene expression were observed (no studies examined body burdens between 250 and 750 ng TEQ/kg). In contrast, longer-term studies in B6C3F1 mice and Sprague-Dawley rats have found ≥4-fold increases in EROD activity at ≥100 ng TEQ/kg. These findings suggest that humans do not respond with detectable induction of enzyme activity at the same dioxin body burden as laboratory rodents (Connor and Aylward 2006).

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 and 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 exposure levels to CDDs are discussed in Section 5.7, Populations with Potentially High Exposures.

There is a limited amount of information available on the toxicity of CDDs in children. Most of the available data come from a series of studies on children living in Seveso during the accidental release of airborne trichlorophenol contaminated with 2,3,7,8-TCDD. Shortly after the accident, early irritative dermal lesions (this effect may not have been related to 2,3,7,8-TCDD exposure) and chloracne were observed in a number of children. Erythema and edema, the main clinical features of the early irritative lesions, were only observed in children and young adults (<20 years old) (Caputo et al. 1988). Chloracne was observed in 187 individuals; 88% of them were children aged 0–14 years (Bisanti et al. 1980). Based on serum 2,3,7,8-TCDD levels measured in 30 Seveso residents with and without chloracne, Mocarelli et al. (1991) suggested that children may develop chloracne at lower 2,3,7,8-TCDD body burdens than adults following acute-duration exposure to 2,3,7,8-TCDD. Other effects observed in the exposed

children include a significant increase in the number of children with chloracne having clinical and electrophysiological signs of peripheral nervous system involvement (assessed 6 years after the accident) (Barbieri et al. 1988) and slight transient increases in serum GGT and ALT levels in boys aged 6–10 years (Mocarelli et al. 1986). Although the serum enzyme levels were higher than in non-exposed children, the values were within the normal range and were elevated 1, 2, and 3 years after the accident, but not after 4 or 5 years. Increased risks of Hodgkin's lymphoma, myeloid leukemia, and thyroid cancer were also reported among children who were 0–19 years old at the time of the Seveso accident (Pesatori et al. 1993). However, the differences in relative risks for these cancer types between the Seveso residents and the control population did not reach statistical significance. Similar results were found in a 15-year follow-up study of this cohort (Bertazzi et al. 1997).

A wide variety of effects have been observed in adults exposed to 2,3,7,8-TCDD at work or following an accidental release of 2,3,7,8-TCDD into the environment. The primary targets appear to be the skin, liver, body weight, and endocrine, reproductive, and immune systems; an increased cancer risk has also been observed. In the absence of data to the contrary, it is likely that these organs/systems will also be sensitive targets in children.

A number of human studies have investigated the potential of 2,3,7,8-TCDD to induce developmental effects. Although some studies have found associations between maternal and/or paternal CDD exposure and developmental effects, particularly for impaired development of the reproductive system, there is no consistent evidence of adverse birth outcomes, thyroid hormone levels, immune effects, or neurodevelopment (see Section 2.17 for additional information).

The toxicity of 2,3,7,8-TCDD has been extensively examined in animal oral toxicity studies, and effects have been observed in most organs/systems (see Section 2.17 for additional information). The animal studies clearly demonstrate that the developing organism is very sensitive to the toxicity of 2,3,7,8-TCDD. The types of effects observed in the offspring of animals exposed to 2,3,7,8-TCDD include fetal/newborn mortality, decreased growth, structural malformations, decreases in birth weight and growth, immunotoxicity, thymic atrophy, impaired development of the reproductive system, and neurodevelopmental effects. The most sensitive developmental effects are impaired development of the reproductive system and neurobehavioral effects.

There is a limited amount of data on the toxicokinetic properties of CDDs in children or immature animals. A toxicokinetic model was constructed that accurately predicted the lifetime concentrations of

2,3,7,8-TCDD in adipose tissue, blood, liver, and feces at different ages (Kreuzer et al. 1997). In formula-fed infants, the model predicted that 2,3,7,8-TCDD lipid levels would decrease during the first year and subsequently increase, reaching a maximum at 16 years of age. In contrast, the model predicted an initial increase in 2,3,7,8-TCDD lipid levels in exclusively breastfed infants followed by a 3-year decrease after weaning and merging at about 7 years with concentrations in formula-fed individuals. The half-life of nonmetabolic elimination (unchanged 2,3,7,8-TCDD) was calculated to be 0.42 years in newborns and 9.5 years in 40-year-old adults. The half-life of the fraction metabolized by the liver ranged from 1.5 years for newborns to approximately 10 years for a 40-year-old individual. The 3 times greater elimination half-life for the metabolized fraction relative to the nonmetabolized fraction in infants suggests that metabolic elimination does not play a major role in the elimination of 2,3,7,8-TCDD in infants. 2,3,7,8-TCDD accumulates preferentially in liver and adipose tissue. Accumulation in the liver is due to sequestration by the microsomal binding protein, CYP1A2. To the extent that this protein is developmentally regulated (Leeder and Kearns 1997), infants (<4 months old) might accumulate relatively less 2,3,7,8-TCDD in their livers than adults. Little is known about the metabolism of 2,3,7,8-TCDD in humans and it is unknown whether the metabolism of 2,3,7,8-TCDD or other CDDs differs between adults and children. In animals, phase II enzymes play an important role in the biotransformation and elimination of 2,3,7,8-TCDD. If this were the case in humans, it would be expected that very young infants would metabolize and eliminate 2,3,7,8-TCDD slower than adults since glucuronosyltransferase activity achieves adult levels by 6–18 months of age (Leeder and Kearns 1997).

CDDs are transferred from mother to offspring through the placenta and human milk. Although there are human data indicating placental transfer of 2,3,7,8-TCDD (Kreuzer et al. 1997; Schecter et al. 1996b), quantitative data are not available. A study in mice administered a single dose of 2,3,7,8-TCDD on GD 12 showed that the rate of accumulation of 2,3,7,8-TCDD in placental tissue reached a maximum in about 3 hours (Abbott et al. 1996); after 24 hours, 0.27% of the maternal dose was detected in the placenta. The transfer of CDDs through the placenta and human milk is discussed in more detail in Section 3.1.4.

CDDs are lipophilic compounds that can concentrate in maternal milk and be transferred to the nursing infant. Numerous studies have examined the transfer of 2,3,7,8-TCDD and related chemicals to infants via human milk and for the most part, the results showed that infants may absorb up to 95% of the administered dose (Abraham et al. 1994, 1996; Dahl et al. 1995; McLachlan 1993; Pluim et al. 1993b). This percentage is similar to the percent of 2,3,7,8-TCDD absorbed (>87%) by an adult volunteer after ingestion of a single oral dose of 2,3,7,8-TCDD (Poiger and Schlatter 1986). As stated previously, it has

also been shown that breastfed infants have a larger 2,3,7,8-TCDD burden during the first year of life compared to formula-fed infants (Kreuzer et al. 1997). However, this initial higher burden does not translate into a higher lifetime burden. A number of human studies have examined breastfed infants of mothers with high background levels of CDDs. These studies have found alterations in some markers of liver, thyroid, and immune function and in neurodevelopment (neurological optimality score) (Huisman et al. 1995a; Koopman-Esseboom et al. 1994; Pluim et al. 1993b, 1994a; Weisglas-Kuperus et al. 1995); however, all of the markers were within the normal range. The impaired neurological optimality score that was observed in newborns was not significantly altered in children aged 6, 18, or 31 months (Ilsen et al. 1996; Huisman et al. 1995b; Pluim et al. 1996).

Subsequent sections of this chapter (Sections 3.3 and 3.4) discuss the available information on biomarkers and interactions. Most of the available information is from adults and mature animals; no child-specific information was identified, with the possible exception of biomarker data. However, there are some data to suggest that interactions with PCBs and CDFs may influence the developmental toxicity of 2,3,7,8-TCDD. Data from children living in Seveso suggest that serum 2,3,7,8-TCDD levels are reflective of exposure levels and are a sensitive indicator of past exposure. Likewise, it is likely that the available information in adults on interactions and methods for reducing toxic effects will also be applicable to children.

As discussed previously, children appear to be unusually susceptible to the dermal toxicity of 2,3,7,8-TCDD. The data are inadequate to assess whether they will also be more sensitive to other CDD effects. Additionally, the available animal data suggest that the developing fetus is very sensitive to 2,3,7,8-TCDD-induced toxicity. 2,3,7,8-TCDD appears to interfere with the development of the reproductive, immune, and nervous systems; the mechanisms of action for these toxic effects have not been elucidated.

Children are primarily exposed to CDDs in the same manner as adults in the general population (i.e., via consumption of foods contaminated with small amounts of CDDs, particularly meat, milk and other dairy products, and fish). Children who are at additional risk of exposure primarily through dietary habits, include: infants and young children who are breastfed; children of recreational and subsistence fishers, who typically consume larger amounts of locally caught fish and shellfish than the general population; children of subsistence hunters, particularly those in the high latitudes, who typically consume large amounts of locally caught game especially marine mammals; and children of subsistence farmers living in

areas contaminated with CDDs (either by waste incinerators or the use of CDD-contaminated sewage on their land) who exclusively consume their own farm-raised beef and dairy products (see Section 5.7).

The human fetus is exposed to CDDs/CDFs through transplacental transfer from the mother. Schecter et al. (1990a) reported 2,3,7,8-TCDD concentrations in liver tissue of three stillborn infants of 0.03–0.18 ppt (whole weight basis) and 1.3–4.3 ppt (lipid weight basis). Schecter et al. (1990a) also reported CDD/CDF concentrations in liver tissue of three stillborn infants of 2.1–4.92 ppt (whole weight basis) and 98–104 ppt (lipid weight basis). The TEQ for CDDs/CDFs combined were 0.14–0.49 ppt (whole weight basis) and 6.4–12 ppt (lipid weight basis). In another study, Schecter et al. (1996c) reported TEQs for CDDs/CDFs in placental material of 8.4–17.6 ppt (lipid basis). In a pooled sample of fetal tissue (8–14 weeks), the TEQ was 5.3 ppt (lipid basis). Concentrations of 2,3,7,8-TCDD in adipose tissue and liver were also reported by Kreuzer et al. (1997) for stillborn babies at levels of 0.2–0.8 and 0.3–0.7 ppt, respectively. Kreuzer et al. (1997) developed a pharmacokinetic model for 2,3,7,8-TCDD that predicted a decrease in body burdens during the first year for non-breastfed infants and this was supported by empirical data (see Section 3.1.4).

In addition to transplacental transfer, CDDs and CDFs have been found in human milk (Fürst et al. 1992; Ryan et al. 1993a; Schecter and Gasiewicz 1987b; Schecter et al. 1986a, 1989b, 1989d, 1989e, 1991a); human milk is thus a potential source of CDDs for nursing infants and children (see Section 5.6). In Binghamton, New York, and Los Angeles, California, human milk was found to contain almost identical levels of detectable CDDs on a lipid basis probably because food consumption and sources are similar across the United States (Schecter et al. 1989e). Mean values of two pooled samples (n=42) from both cities showed that OCDD was the most abundant congener present (233 ppt), followed in decreasing order by total HxCDD (42.65 ppt), 1,2,3,4,6,7,8-HpCDD (42 ppt), 1,2,3,6,7,8-HxCDD (30.5 ppt), 1,2,3,7,8-PeCDD (6.7 ppt), 1,2,3,7,8,9-HxCDD (6.2 ppt), 1,2,3,4,7,8-HxCDD (4.95 ppt), and 2,3,7,8-TCDD (3.3 ppt). The total CDDs value was reported as 327 ppt. The TEQ for CDDs/CDFs, but not PCBs, in human milk in the United States was 17 ppt (Schecter et al. 1989e). Between 1986 and 1987, concentrations of CDDs found in human milk sampled from Canadian women ranged from 2.2 ng/kg (ppt) (lipid basis) for TCDDs to 173 ppt for OCDD. In addition, the combined CDD/CDF mean TEQ of 15.6 ppt (lipid basis) declined from a TEQ of 24.7 ppt measured in 1981–1982 (Ryan et al. 1993a).

CDD/CDF concentrations also have been measured in human milk in several foreign studies. The levels of CDDs were 5.3–139.5 ng TEQs/kg milk fat in studies of women in the Netherlands, Canada, Germany,

Siberia, United Kingdom, South Vietnam, and Cambodia (Dewailly et al. 1991; Duarte-Davidson et al. 1992; Fürst et al. 1994; Pluim et al. 1994a). Transplacental transfer of CDDs has also been demonstrated in humans (Kreuzer et al. 1997; Pedersen et al. 2010). OCDD was a major component in human milk (50.2–494.0 ng/kg milk fat) and the concentrations tended to decrease with the degree of chlorination. CDD concentrations in human milk can be directly correlated with the age of the mother and the amount of animal (but not vegetable) fat and protein consumed, suggesting that meat, milk and other dairy products, and fish are the major sources of CDD intake (Pluim et al. 1993a). The fact the CDD concentrations in milk fat were significantly related to age is in agreement with the results of Stanley et al. (1986) and Orban et al. (1994) who reported a strong correlation between age group and CDD levels in adipose tissue in the general U.S. population. The positive correlation can be expected because of the long half-life of CDDs in humans (7–11.3 years) (Pirkle et al. 1989; Wolfe et al. 1994).

Estimated daily intakes of CDD/CDF TEQs by nursing infants in the United States have been reported by Schecter and Gasiewicz (1987a). The daily intake by nursing infants in the United States was estimated to be 83.1 pg TEQs/kg body weight/day. To determine this daily intake, various assumptions were made regarding infant body weight (10 kg), duration of nursing, average amount of milk consumed, and gastrointestinal absorption. It was also assumed that human milk was the only source of CDDs while the infant was nursing during the first year of life. From results of earlier studies that determined the concentrations of CDDs/CDFs in human milk in the United States (Schecter et al. 1989e) and in cow's milk and soybean-derived infant formula sold in the United States (Schecter et al. 1989c) (see Section 5.5.4), Schecter et al. (1994a) estimated intakes of 35–53 pg TEQ/kg of body weight/day for infants (7.3 kg) who were breastfed within the first year of life as compared to 0.07–0.16 pg TEQ/kg of body weight for infants who were fed soy formula.

Exposure of infants and young children to CDDs may be very high because of their relatively high consumption of milk, including human milk (ECETOC 1992). Schecter et al. (1994a) evaluated the intake of CDDs/CDFs from human milk and estimated that high levels reported for human milk in the United States (≈17 ppt TEQ on a lipid basis) contribute 35–53 pg TEQ/kg of body weight per day to the nursing infant in its first year of life (Schecter et al. 1989e). The CDD concentrations in cow's milk and soy-based formula were much lower than the 327 ppt concentration in human milk (Schecter et al. 1991a). The following concentrations for CDDs (on a lipid basis) were reported: cow's milk (25.1 ppt), 2% cow's milk (32.3 ppt), Similac™ infant formula (39 ppt), Isomil™ infant formula (23.3 ppt), and Prosobee™ infant formula (42.7 ppt) (Schecter et al. 1989c). The TEQ values for cow's milk and soy-based infant formula were also much lower than for human milk (≈17 ppt). The corresponding TEQ

values for CDDs/CDFs (on a lipid basis) were reported: cow's milk (2.1 ppt), 2% lowfat cow's milk (0.79 ppt), Similac™ infant formula (0.08 ppt), Isomil™ infant formula (0.05 ppt), and Prosobee™ infant formula (0.127 ppt) (Schecter et al. 1989c). Schecter and Gasiewicz (1987a, 1987b) calculated TEQ values for CDDs/CDFs in human milk in two populations in Vietnam and in the general population in the United States. The study authors reported mean values during the 1980s of 1.04 pg TEQ/g (whole milk basis) for the United States (maximum 4.72 pg TEQ/g), a mean of 1.11 pg TEQ/g for South Vietnamese (maximum value 4.38 pg TEQ/g) exposed to Agent Orange sprayed between 1962 and 1970, and a mean of 0.065 pg TEQ/g (maximum value 0.18 pg TEQ/g) for a North Vietnamese population that was not exposed to Agent Orange. The study authors concluded that some infants in the United States (whose mothers had CDD milk concentrations in the upper range of measured values) were being exposed to mean concentrations comparable to levels observed in the South Vietnamese population exposed to Agent Orange (Schecter and Gasiewicz 1987a, 1987b).

The highest exposure to CDD-contaminated human milk reported was associated with the widespread use of Agent Orange as a defoliant during the Vietnam War. Human milk specimens from Ho Chi Minh City and Song Be Province in South Vietnam had lower 2,3,7,8-TCDD values in the late 1980s (7.1 and 17 ppt lipid basis, TEQ values of 18.5 and 31.7 ppt, respectively) than they did in the 1970s when Agent Orange spraying occurred (Schecter et al. 1989e). A 1970 mean value for 2,3,7,8-TCDD in human milk in South Vietnam was reported to be 484.9 ppt (range, not detectable to 1,450 ppt) (Baughman and Meselson 1973; Schecter et al. 1986a). These values serve as reference values for the highest levels of 2,3,7,8-TCDD documented in human milk (Schecter et al. 1989e). Estimated daily intakes of TEQs by nursing infants from Vietnam have been reported (Schecter and Gasiewicz 1987a). The estimated daily intake by nursing infants in southern Vietnam in 1970 was 908 pg TEQs/kg body weight/day, whereas the daily intakes in southern and northern Vietnam in 1984 were 88.7 and 5.1 pg TEQs/kg body weight/day, respectively. Analysis of nine milk samples from individuals living in northern Vietnam showed no detectable concentrations of 2,3,7,8-TCDD (detection limit 2 ppt) (Schecter and Gasiewicz 1987a). To determine these daily intakes, various assumptions were made regarding infant weight, duration of nursing, average amount of milk consumed, and gastrointestinal absorption. It was also assumed that human milk was the only lifetime source of exposure to CDDs during the first year of life. In another study, Tarkowski and Yrjanheikki (1989) evaluated the health risks associated with human milk. The study authors concluded that levels of CDDs/CDFs in human milk did not present a health risk to infants or children and that there was no justification for limiting breastfeeding. However, the study authors believed that there was a need for primary prevention of CDD/CDF exposure in humans. Because of the relatively short period of intake and the accepted benefits of breastfeeding, WHO (1991) did not

recommend limitations on breastfeeding at the levels of background exposures to CDDs and CDFs. Pohl and Hibbs (1996) reviewed studies indicative of a possible link between development of subtle health effects in children and their exposure to CDDs and CDFs from maternal milk. It is the ATSDR position that for background exposures, the benefits of breastfeeding outweigh any potential risk associated with exposure. For higher CDDs levels in human milk, the safety of breastfeeding may be of concern in some cases.

Two studies have looked at ways to reduce CDD exposure in breastfed infants. Koppe (1995) reported that exposure before and after birth to CDDs and PCBs has given rise to subtle abnormalities (disturbed cognitive development and delayed motor development) in approximately 10% of newborns in the Netherlands. The study author examined possibilities of reducing this exposure by influencing the diet of the lactating mother. Mobilization of fatty acids from adipose tissue will cause release of stored CDDs, which will then be secreted in human milk. Two maternal diets were tested for their ability to reduce concentrations of CDDs in human milk. One diet was a low-fat/high-carbohydrate/low-CDD diet, while the second was a high-fat/low-carbohydrate/low-CDD diet. Despite significant changes in fatty acid profiles of the milk, no significant changes in CDD concentrations in human milk were observed. The study author concluded that short-term dietary measures will not reduce CDDs in human milk. A lowering of CDD intake must occur years before the woman becomes pregnant. An important food source for the women is cow's milk and other dairy products and these are responsible for about half of the daily exposure CDDs and PCBs in women in the Netherlands, so levels of the compounds in dairy foods must be lowered. In addition, the study author believed that a lowering of CDD concentrations in fish is also necessary. Based on the results of his dietary study, Koppe (1995) reported that daily dietary intake of CDDs during lactation represents only 14% of the daily secretion of CDD in human milk, while 86% was derived from CDDs stored in adipose tissue. Thus, reducing dietary intake of CDDs during lactation would only reduce CDDs in milk by 14%. Schlaud et al. (1995) also reported that to reduce organochlorine residue levels, including CDDs in human milk in the short-term, nursing mothers should be advised not to try to reduce their body weight until after lactation. The study authors reported statistically significant positive associations between human milk contamination and average dietary fat intake per week ($p=0.001$) and proximity of residence to hazardous waste sites ($p<0.05$) for CDDs. The study authors believe that public promotion of a lower dietary fat intake may reduce the lifetime accumulation of CDDs in human fatty tissues and in the long-term, resulting in lower concentrations in human milk as well.

In addition to exposure to CDDs through consumption of human milk, cow's milk, and soy-based infant formula, older children can be exposed through dietary practices similar to those of adults in the general population (see Section 5.5.4). One study looked at the exposure that might occur in a 6-year-old child who consumes "fast foods." In 1995, Schecter and Li (1997) conducted a congener-specific analysis of CDDs, CDFs, and dioxin-like PCBs in U.S. fast foods. The study authors reported CDD/CDF TEQ values, depending on the treatment of not detected congeners, of 0.03–0.28 pg/g wet weight for one McDonald's Big Mac, 0.03–0.29 pg/g for one Pizza Hut personal pan pizza supreme with all toppings, 0.01–0.49 pg/g for one Kentucky Fried Chicken three-piece original recipe meal, and 0.3–0.31 pg/g for one Häagen-Daz chocolate-chocolate chip ice cream. The daily intake from one serving of each of the fast foods tested, assuming a 20-kg child (6 years old), ranged between 0.15 and 5.05 pg TEQ/kg body weight. The study authors calculated that, on average, a child (6 years old) consumes 3 times more TEQs on a per kg/body weight basis than an adult eating any one of the fast foods tested.

As a result of the transfer of CDDs through the placenta to the fetus, by human milk to infants and young children, and by lifelong dietary intakes from the consumption of meat, milk and dairy products, and fish, CDDs are found to be widespread in the adipose tissue of members of the general population (Orban et al. 1994). Human adipose samples from the 1987 National Human Adipose Tissue Survey (NHATS) provide a representative sample of CDD body burden in the general U.S. population (see Section 5.6). The average concentration of 2,3,7,8-TCDD in the U.S. population was estimated to be 5.38 pg/g (±6%). The 1987 survey data clearly show, however, that nearly all the CDD/CDF congeners in adipose tissue increased with the age of the donor (i.e., the highest concentrations occurred in the ≥45-year-old age group and the lowest concentrations occurred in children in the 0–14-year-old age group). The average concentration of 2,3,7,8-TCDD in the 1987 survey increased from 1.98 pg/g in the 0–14-year-old group, to 4.37 pg/g in the 15–44-year-old group, to 9.4 pg/g in the ≥45-year-old group.

Children may be exposed to CDDs through a variety of lifestyle practices of their parents or of their own. For example, CDD/CDF concentrations have been reported in cigarette smoke (Lofroth and Zebuhr 1992; Muto and Takizawa 1989) (see Section 5.5.4). Young children and infants may be exposure to CDDs indirectly by inhalation of room air contaminated from cigarette smoking of their parents. In addition, older children and teenagers may be directly exposed if they become smokers themselves. Malisch (1994) reported that some colored candle wax produced with certain dye pigments contained CDDs/CDFs. By burning these candles, CDDs could be released into room air and be an additional source of inhalation exposure for children.

Children may also be exposed to CDDs by dermal contact with some new, unwashed clothing, particularly those manufactured in some developing countries or from fabric shipped from developing countries where PCP is used for preserving cotton fabrics during sea transport (Horstmann and McLachlan 1994). Exposures can be reduced by washing new clothes prior to wearing.

Children could potentially be exposed to CDDs at home from a variety of incineration sources. For example, if their parents routinely burn domestic garbage containing scrap wood treated with PCP (Chiu et al. 1983) or untreated wood (Clement et al. 1985), have old pesticide containers that may have contained 2,4,5 T or 2,4-D or Silvex (Arthur and Frea 1989), have PVC pipes or other plastics items (Lustenhouwer et al. 1980), or extensively use a wood stove (Clement et al. 1985), children may be exposed to higher levels of CDDs in outdoor and/or indoor air. Time spent in a garage where cars or trucks are being repaired and the engines are running exposes children and teenagers to exhaust products and engine soot that may also contain CDDs (Bingham et al. 1989; Cirnies-Ross et al. 1996).

Although there are many studies on the effects of CDDs on adults who receive occupational exposures (Fingerhut et al. 1989; Hesso et al. 1992; Patterson et al. 1989a; Schecter et al. 1985a, 1994b; Tepper et al. 1997), no information was located on the potential for workers in the United States to bring CDDs home on their clothing or shoes, thus contaminating other family members, including children. It is conceivable, however, that because CDDs are present in a variety of diverse occupational settings (see Section 5.6), poor occupational hygiene could result in CDDs being brought home and contaminating domestic dwellings.

Children in populations with potentially high exposure living in the vicinity of former or current production sites where CDDs are released as byproducts (e.g., incinerators, other waste disposal facilities, and hazardous waste sites) may be exposed to CDDs by several pathways (see Section 5.7). Children may be exposed to CDDs in CDD-contaminated soils. Dermal absorption from contaminated soil, however, is likely to be inefficient (Poiger and Schlatter 1980; Shu et al. 1988; Weber et al. 1991b). Young children are potentially exposed to CDDs because of their tendency, through hand-to-mouth activity, to ingest soils (pica) that may be contaminated with CDDs (see Section 5.7 for further details) (Fries and Paustenbach 1990; Kimbrough et al. 1984; Paustenbach et al. 1992; Pohl et al. 1995). LaGoy (1987) estimated the following average soil ingestion rates for children: 0–1 year old, 50 mg/day (maximum 250 mg/day); 1–6 years old, 100 mg/day (maximum 500 mg/day); 6–11 years old, 50 mg/day (maximum 250 mg/day); and >11 years old, 50 mg/day (maximum 100 mg/day). If children ingest

between 50 and 100 mg of soil per day (LaGoy 1987) and the soil that they ingest contains 1 pg/g (1 ppt) of CDDs, a child may be exposed to 0.05–0.1 pg CDDs/day by this pathway alone (see Section 5.7).

Children in high-risk populations include children of recreational or subsistence fishers, children of subsistence hunters particularly those who consume tissues of marine mammals, and children of subsistence farmers who consume meat, milk, and/or dairy products from their own farm-raised animals (see Section 5.7 for further details). For example, Native American and other subsistence fishing communities may be at greater health risks from CDDs in fish, and children in these population often consume larger amounts of fish than adult members of the general population (CRITFC 1994; Mott 1995). Children of recreational and subsistence fishers who routinely consume locally caught fish from CDD-contaminated waterbodies can be exposed to higher CDD concentrations than children who consume similar or larger amounts of commercially marketed fish from a variety of sources (Ebert et al. 1996; EPA 1995; Mott 1995). The exposure to CDDs will also be highest among children who regularly eat fish as compared to those who only occasionally eat fish or never eat fish. Several studies have documented the higher fish consumption rates among subsistence fishers, some of which are Native American populations (CRITFC 1994; Nobmann et al. 1992; Wolfe and Walker 1987). A study of fish consumption patterns among the Umatilla, Nez Perce, Yakama, and Warm Springs tribes of the Columbia River Basin in Washington and Oregon (CRITFC 1994) found that the consumption rate for these Native American children (≤5 years) from these four tribes was 19.6 g/day (a consumption rate over 3 times higher than that for adults in the general population [6.5 g/day]).

This increased exposure has been demonstrated by serum CDD levels that are found to be several times higher in people who regularly eat fish as compared to those who occasionally eat fish or never eat fish (Anderson et al. 1998; Svensson et al. 1991) (see Sections 5.7). In addition, this same situation also applies for consumption of wildlife, specifically marine mammals (Ayotte et al. 1997; Dewailly et al. 1992). Similar dietary situations exist for children of subsistence hunters who tend to consume tissues of marine mammals and children of subsistence farmers who consume beef, milk, and other dairy products from their own farm-raised animals. In the case of subsistence fishers, subsistence hunters, and subsistence farmers, all three populations share one problem, that the sources of their fish, meat, and/or milk and other dairy products are typically restricted to a localized area, and if these food sources are contaminated with CDDs, adults and children in these populations will be exposed to higher levels of CDDs than members of the general population (see Section 5.7 for additional details on these populations at risk).

In order to reduce exposure from consumption of CDD-contaminated fish and wildlife, consumption advisories are issued by states recommending that individuals restrict their consumption of specific fish, shellfish, and wildlife species from certain waterbodies where CDD concentrations in tissues of these species exceed the human health level of concern (EPA 1995) (see Section 5.7 for additional information). Recreational and subsistence fishers typically consume larger quantities of fish and shellfish than the general population and frequently fish the same waterbodies routinely. Because of this, children living in these populations are at greater risk of exposure to CDDs and other chemical contaminants if the waters they fish are contaminated. EPA (1998b) reported that 66 advisories have been issued by 21 states restricting the consumption of CDD-contaminated fish and shellfish, and one state Arkansas also issued a consumption advisory for wood ducks, a species of migratory waterfowl. Three states (New Jersey, New York, and Maine) also had statewide advisories for CDDs in their marine waters (EPA 1998a).

As reviewed by Connor and Aylward (2006), a number of AhR polymorphisms (defined as an allelic frequency of >1% in a given population) have been identified. However, correlations between the observed human AhR genotype and CYP1A/B inducibility have not been established. Connor and Aylward (2006) noted that because the AhR has been shown to have a critical role in development and homeostasis, there is little tolerance for genetic variations, other than those that are inconsequential to AhR function. Human polymorphisms frequently occur in exon 10, a region that encodes a major portion of the transactivation domain of the AhR that is responsible for regulating the expression of other genes (e.g., CYP1A1) (Harper et al. 2002). Variation that is confined to the transactivation domain may permit finely tuned modulation in gene regulation without abolishing the critical roles of AhR in development and homeostasis (Harper et al. 2002). Most of the ‘defective’ phenotypes that have been identified in human cells or tissues are in the direction of non-responsiveness or low inducibility. Only one pair of human polymorphisms, those at codons 517 and 570, has been shown to have a clear-cut and strong effect on the phenotype of an AhR-mediated response.

A wide range of AhR binding capacities has been measured in humans, and a number of investigators have interpreted this range of dissociation constants as indicating a heterogeneous human AhR with functionally important polymorphisms (Connor and Aylward 2006). However, some of the observed variation may be due to experimental factors (differences in composition/cellular makeup of the samples) and environmental and dietary influences. Studies on human placental tissues have found at least a 10-fold range of AhR binding affinities for 2,3,7,8-TCDD. However, sequencing the AhR complimentary DNA (cDNA) from a few individuals with the highest and lowest affinities did not reveal

any polymorphisms that would explain the variation in ligand binding (Harper et al. 2002). Although polymorphisms on the AhR that would influence normal receptor function are unlikely, genetic variations might exist in non-AhR components such as ARNT, AhR repressor (AhRR), co-activators, or co-repressors, which may affect AhR-mediated events. However, the possible variations have not been fully explored (Harper et al. 2002).

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 2006).

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 CDDs from this report are discussed in Section 5.6, General Population Exposure.

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 2006). 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 CDDs 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 CDDs 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 2006). 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 CDDs 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

CDDs are ubiquitous environmental contaminants that have been measured in biological fluids and tissues of the general population. Adipose tissue and the liver are the primary storage sites for CDDs. It was demonstrated that the relative (lipid-based) levels of 2,3,7,8-TCDD are similar in hepatic and adipose tissues (Leung et al. 1990a) and between adipose tissue and serum (Patterson et al. 1988; Schecter et al. 1990b) from the same patients. Thus, measurement of 2,3,7,8-TCDD levels in serum lipids is considered an accurate and practical measure of body burden. However, this was not the case for more highly chlorinated dioxins; for example, for OCDD, there is a 2:1 ratio between serum and adipose tissue lipid fractions (Schecter et al. 1990b) and a 12:1 ratio between liver and adipose tissue levels (Thoma et al. 1990). The important TEQ variable was close to a 1:1 ratio. CDDs have also been detected in human milk of women exposed to high levels of CDDs and in women presumably exposed to background levels. How human milk levels relate to CDD exposure or body burden has not been established; both parity and the length of time the woman has been lactating influence the CDD concentration in human milk.

The half-lives of CDDs have been estimated from blood samples of highly exposed individuals (workers, Operation Ranch Hand veterans, and the Seveso cohort). The half-lives of 2,3,7,8-TCDD range from 5.8 to 8.7 years (Aylward et al. 2013; Flesch-Janys et al. 1996; Michalek et al. 1996; Needham et al. 1994; Ott and Zober 1996; Yamamoto et al. 2015b). Less information is available of other CDD congers; estimated half-lives of 13.8–15.7 years for 1,2,3,7,8-PeCDD, 8.4–10.7 years for 1,2,3,4,7,8-HxCDD, 9.0–13.1 years for 1,2,3,6,7,8-HxCDD, 4.8–6.3 years for 1,2,3,7,8,9-HxCDD, 6.7–3.7 years for 1,2,3,4,6,7,8-HpCDD, and 6.7–7.3 years for OCDD have been reported (Aylward et al. 2013; Flesch-Janys et al. 1996; Yamamoto et al. 2015b). Aylward et al. (2013) and Yamato et al. (2015b) also estimated half-lives of 8.7–9.1 years for total TEQ (CDDs, CDFs, and dioxin-like PCBs). Information on the levels of CDDs in biological tissues is presented in Sections 5.6 and 5.7.

3.3.2 Biomarkers of Effect

Chloracne is one effect that is clearly associated with exposure to high levels of CDDs and other halogenated organic chemicals and has been observed in some individuals who were exposed occupationally or in the environment to increased levels of 2,3,7,8-TCDD or chemicals contaminated with 2,3,7,8-TCDD. However, while the presence of chloracne indicates exposure to CDDs or other halogenated organic compounds, its absence does not preclude such exposure. For example, in a cohort from the Seveso incident, no chloracne was observed below an initial serum lipid 2,3,7,8-TCDD level of 800 ppt (body burden of 2.5 μg/kg, assuming 22% body fat and 70 kg body weight); above 12,000 ppt (body burden of 38 μg/kg), chloracne was always observed; and between 800 and 12,000 ppt, the occurrence of chloracne was sporadic (Mocarelli et al. 1991). In the Yu-Cheng population, chloracne was associated with a body burden in 2,3,7,8-TCDD equivalents of 2–3 μg/kg body weight, or about 140–210 μg for a 70-kg adult (Ryan et al. 1990).

Biochemical changes (raised serum hepatic enzyme levels, disorders of lipid and carbohydrate metabolism, unbalanced porphyrin metabolism) and/or an enlarged liver can indicate effects induced by 2,3,7,8-TCDD exposure, but these effects are not specific for this or other compounds. Light and electron microscope changes in the liver (e.g., lipid droplets in parenchymal cells, increased endoplasmic reticulum, enlarged and pleomorphic mitochondria) are also sensitive but nonspecific biomarkers for exposure to CDDs (Schecter et al. 1985b). When biochemical changes in the placenta of women exposed in the Yu-Cheng incident were evaluated for use as possible biomarkers, the EGF receptor autophosphorylation effect was found to be associated with decreased birth weight in the neonates (Lucier et al. 1986). The study authors suggested using this response as a biomarker of effect for all toxic chlorinated aromatic compounds.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Several studies were located regarding interactions that affect the toxicity of CDDs. Probably the most important interactions that have an impact on human health are those involving CDDs, CDFs, and PCBs. It has been recognized that chloroaromatics cause a complex of similar effects that vary in severity depending on the number of chlorine atoms, positional substitution, and species susceptibility. Sufficient information is available for assessment of risk associated with exposure to 2,3,7,8-TCDD. However, exposure to a mixture of chloroaromatics is common in the general environment. The assessment of

health risk resulting from exposure to chemical mixtures of chloroaromatics was enabled by the development of TEFs (2,3,7,8-TCDD equivalence factors) that relate the relative toxic potency for CDDs and CDFs to that of 2,3,7,8-TCDD (EPA 1989). It was assumed based on previous literature data (Eadon et al. 1986) and in animal dosing studies (Van den Berg et al. 1989), that CDDs and CDFs have an additive effect in the organism when weighted for relative toxicity compared to 2,3,7,8-TCDD (for further information see Sections 2.1). The assumption of additivity was later supported by experimental data. The concept of TEFs was used, for example, to assess the potential toxicity of background levels of CDFs and CDDs in general populations based on body burdens of indicator CDDs that were associated with chloracne and other effects in the Yusho and Yu-Cheng rice oil poisoning incidents (Ryan et al. 1990).

However, some studies further investigated the interactions of various chloroaromatics and indicated that the interactions may be more complicated. *In vitro* studies compared relative toxicity of various chloroaromatics in human cell lines monitoring enzyme induction and binding to the AhR that mediates the induced responses (Nagayama et al. 1985; Safe 1987). *In vivo* studies concentrated on monitoring enzyme induction, inhibition of body weight gain, and immunotoxic and teratogenic effects. Coexposure of Long-Evans rats to 6-methyl-1,3,8-trichlorodibenzofuran (MCDF) and 2,3,7,8-TCDD induced a partial inhibition of the monooxygenase enzyme-induction response caused by 2,3,7,8-TCDD treatment alone (Harris et al. 1989). Although MCDF did not decrease the levels of occupied nuclear 2,3,7,8-TCDD AhRs, it inhibited the effects of 2,3,7,8-TCDD on the cytosolic AhR (Harris et al. 1989).

Other studies further indicated that PCBs may antagonize AhR-mediated responses to 2,3,7,8-TCDD. In a review, Van den Berg et al. (1994) suggested that toxicokinetic factors contribute to the observed nonadditive toxicological and biological effects. Co-treatment of C57BL/6 mice with various commercial Aroclors (PCB mixtures) and 2,3,7,8-TCDD resulted in antagonizing the 2,3,7,8-TCDD-mediated inhibition of the splenic plaque-forming cell response (Bannister et al. 1987; Davis and Safe 1989). Similarly, significant antagonism of 2,3,7,8-TCDD and Aroclor 1254 was observed in the induction of CYP-dependent monooxygenases in C57BL/6J mice (Bannister et al. 1987). The effects were dependent on the dose of both 2,3,7,8-TCDD and Aroclor 1254 and on their respective ratios. The ratios of Aroclor 1254/2,3,7,8-TCDD that induced antagonist reactions were comparable to the ratios of PCBs/CDDs found in human tissues and environmental samples. The study authors speculated that less-toxic chlorinated compounds may have a protective effect against the more-toxic compounds in the environment. However, by comparing the immune sensitivities of both Ah-responsive and Ah-less-responsive mouse strains, it was demonstrated that a complex mixture of contaminants taken from the Love Canal site was immunosuppressive and that this effect was primarily due to the 2,3,7,8-TCDD component of the

mixture, although 2,3,7,8-TCDD was a very minor component and there was little interaction with the other hydrocarbon components of the mixture (Silkworth et al. 1989a).

Experimental studies have shown that interactions of 2,3,7,8-TCDD and CDFs or PCBs resulted in fetotoxic and teratogenic effects in the offspring of exposed animals. Exposure of pregnant mice to 2,3,7,8-TCDF resulted in cleft palates and hydronephrosis in the offspring (Hassoun et al. 1984). The results obtained in different strains of mice indicated an association with the Ah locus. Comparable results were obtained previously in mice exposed to 2,3,7,8-TCDD (Abbott and Birnbaum 1989a; Abbott et al. 1987a, 1987b; Courtney 1976). When C57BL/6N mice were treated orally with 2,3,7,8-TCDD and 2,3,7,8-TCDF on GD 10, hydronephrosis and cleft palates were observed in the offspring (Weber et al. 1985). The effects of both chemicals were additive. Similarly, an increased incidence (10-fold) of cleft palates was observed in offspring of C57BL/6N mice after a combined treatment with 2,3,7,8-TCDD and 2,3,4,5,3',4'-hexachlorobiphenyl during gestation, as compared with those treated with 2,3,7,8-TCDD alone (cleft palate was not observed when 2,3,4,5,3',4'-hexachlorobiphenyl was administered alone) (Birnbaum et al. 1985). In contrast, no potentiation of CDD-mediated effect was found with 2,4,5,2',4',5'-hexachlorobiphenyl. Furthermore, co-treatment of pregnant C57BL/6J mice with Aroclor 1254 and 2,3,7,8-TCDD resulted in a sharp decrease in the incidence of cleft palate per litter (8.2%) compared with those treated with 2,3,7,8-TCDD alone (62%) (Haake et al. 1987).

Similarly, 2,3,7,8-TCDD-induced fetotoxicity and teratogenicity were altered by co-exposure to other chemicals. A synergistic effect on the induction of cleft palates was observed in offspring of C57BL/6N mice treated orally with 2,3,7,8-TCDD and retinoic acid on GD 10 or 12 (Abbott and Birnbaum 1989b; Birnbaum et al. 1989b). However, the co-administration of retinoic acid did not influence the incidence of 2,3,7,8-TCDD-induced hydronephrosis, nor did 2,3,7,8-TCDD affect the incidence or severity of limb-bud defects induced by retinoic acid (Birnbaum et al. 1989b). A synergistic effect was observed when 2,3,7,8-TCDD (orally) and hydrocortisone (subcutaneously) were administered to C57BL/6N mice on GDs 10–13 (Birnbaum et al. 1986). The incidence of cleft palate in the offspring increased to 100% following the combined treatment. Pretreatment of pregnant NMRI mice with benzo(*a*)pyrene subcutaneously 5 hours prior to an intraperitoneal injection of 2,3,7,8-TCDD caused an increase in CDD-induced lethality but did not alter the rate of cleft palate formation (Hassoun 1987). Offspring of male mice, treated with chlorinated phenoxy acids and 2,3,7,8-TCDD in their feed for 8 weeks before the mating, did not differ in their development or survival from offspring in the control group (Lamb and Moore 1981).

Results in B6C3F1 mice indicated that α-naphthoflavone antagonizes 2,3,7,8-TCDD in induction of splenocyte EROD activity (Blank et al. 1987). It was further suggested that α-naphthoflavone impedes 2,3,7,8-TCDD suppression of B lymphocyte differentiation by competing for binding to the AhR. The mechanism of interaction of these chemicals was studied *in vitro* using rat hepatic cytosol or mouse hepatoma cells (Gasiewicz and Rucci 1991). The results indicated that α-naphthoflavone acts as a 2,3,7,8-TCDD antagonist by binding to the AhR and forcing on it a conformation that cannot identify the DNA recognition sequence contained in the dioxin-responsive enhancer element of the CYP1A1 gene. In contrast, *in vitro* experiments showed that co-exposure of a thymus organ culture with the weakly toxic β-naphthoflavone and 2,3,7,8-TCDD results in a significant increase in the lymphoid inhibitory effect mediated by 2,3,7,8-TCDD (Hassoun 1987). A developmental toxicity study in mice administered 28 µg/kg 2,3,7,8-TCDD on GD 10 demonstrated that administration of 5 or 5,000 µg/kg α-naphthoflavone significantly reduced the incidence of cleft palate (Yuan et al. 2017). This study also found that administration of 5 mg/kg folic acid also decreased the incidence of cleft palate.

Hexachlorobenzene acted like a weak AhR agonist and caused an up to 40% decrease in specific hepatic cytosol binding of 2,3,7,8-TCDD in rat cells (Hahn et al. 1989). Similarly, 2,3,7,8-TCDD-induced myelotoxicity and enzyme induction were antagonized by 1-amino-3,7,8-trichlorodibenzo-*p*-dioxin in B6C3F1 mice, presumably by competitive binding to the cytosolic AhR (Luster et al. 1986). Comparable effects were observed *in vitro* in murine bone-marrow-cell cultures. Treatment of Fischer-344 rats orally with di(2-ethylhexyl)phthalate (DEHP) before or after oral administration of 2,3,7,8-TCDD reduced the hyperlipidemia induced by the latter compound (Tomaszewski et al. 1988). Furthermore, DEHP pretreatment followed by daily doses of this hypolipidemic substance was partially protective against 2,3,7,8-TCDD-induced mortality, wasting, and liver fatty changes.

The addition of activated charcoal or dehydrocholic acid to the feed, protected animals (C57BL/6J mice, CD-COBS rats, and guinea pigs) from increased mortality caused by a single lethal dose of 2,3,7,8-TCDD (Manara et al. 1984). In the case of the former agent, the effect was probably due to the general high binding ability of superactivated charcoal; since no other antidote is known, its use for therapeutic purposes was recommended. Protective effects of ascorbic acid (administered orally) and butylated hydroxyanisole (BHA) (administered orally) against 2,3,7,8-TCDD given by gavage were investigated in Sprague-Dawley rats (Hassan et al. 1987). BHA administration partially protected rats from losses in organ weights and 2,3,7,8-TCDD-induced lipid peroxidation and inhibition of glutathione peroxidase activity. In contrast, ascorbic acid had no protective effects.

Data regarding interactions affecting the toxicity or toxicokinetics of other chemicals by 2,3,7,8-TCDD were limited. Dermal pretreatment with 2,3,7,8-TCDD inhibited the induction of skin tumors by subsequently applied benzo(*a*)pyrene or dimethylbenz(*a*)anthracene in Sencar mice (Cohen et al. 1979). It was proposed that 2,3,7,8-TCDD caused qualitative alteration of hydrogen binding to DNA. In addition, 2,3,7,8-TCDD may also promote the metabolism of procarcinogens (e.g., 3-methylcholanthrene) to active metabolites by the induction of metabolizing enzymes (Kouri et al. 1974, 1978).