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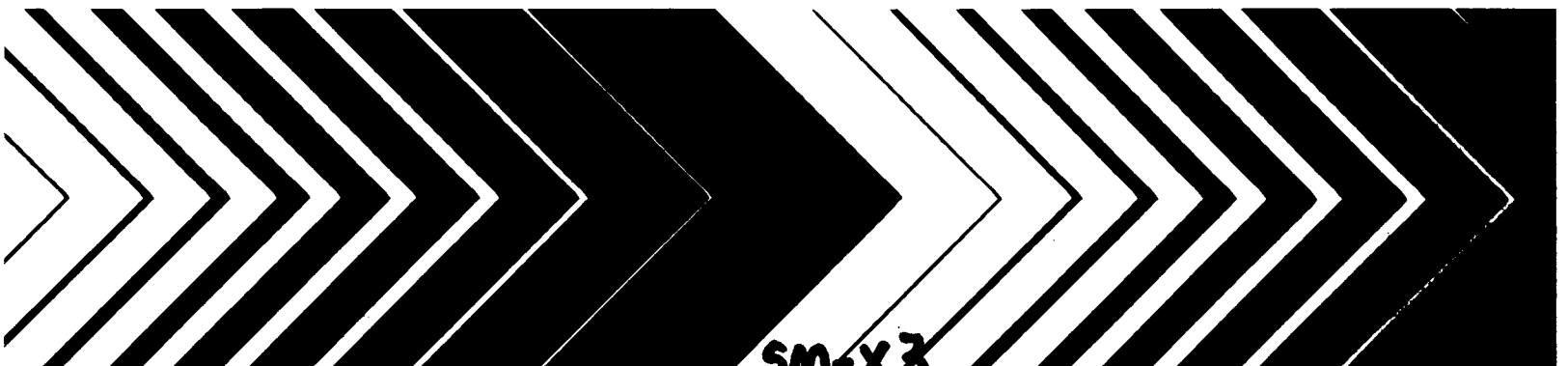

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Chapter 1. Disposition and Pharmacokinetics

Health Assessment for
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)
and Related Compounds

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Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, D.C.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
Ah	Aryl hydrocarbon
AHH	Aryl hydrocarbon hydroxylase
ALT	L-alanine aminotransferase
AST	L-asparate aminotransferase
BDD	Brominated dibenzo-p-dioxin
BDF	Brominated dibenzofuran
BCF	Bioconcentration factor
BGG	Bovine gamma globulin
bw	Body weight
cAMP	Cyclic 3,5-adenosine monophosphate
CDD	Chlorinated dibenzo-p-dioxin
cDNA	Complementary DNA
CDF	Chlorinated dibenzofuran
CNS	Central nervous system
CTL	Cytotoxic T lymphocyte
DCDD	2,7-Dichlorodibenzo-*p*-dioxin
DHT	5α-Dihydrotestosterone
DMBA	Dimethylbenzanthracene
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid

DRE	Dioxin-responsive enhancers
DTG	Delayed type hypersensitivity
DTH	Delayed-type hypersensitivity
ED_{50}	Dose effective for 50% of recipients
ECOD	7-Ethoxycoumarin-0-deethylase
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
EROD	7-Ethoxyresurofin 0-deethylase
EOF	Enzyme altered foci
FSH	Follicle-stimulating hormone
GC-ECD	Gas chromatograph-electron capture detection
GC/MS	Gas chromatograph/mass spectrometer
GGT	Gamma glutamyl transpeptidase
GnRH	Gonadotropin-releasing hormone
GST	Glutathione-S-transferase
HVH	Graft versus host
HAH	Halogenated aromatic hydrocarbons
HCDD	Hexachlorodibenzo-p-dioxin
HDL	High density lipoprotein
HxCB	Hexachlorobiphenyl
HpCDD	Heptachlorinated dibenzo-p-dioxin
HpCDF	Heptachlorinated dibenzofuran

LIST OF ABBREVIATIONS (cont.)

HPLC	High performance liquid chromatography
HRGC/HRMS	High resolution gas chromatography/high resolution mass spectrometry
HxCDD	Hexachlorinated dibenzo-p-dioxin
HxCDF	Hexachlorinated dibenzofuran
ID_{50}	
I-TEF	International TCDD-toxic-equivalency
LD_{50}	Dose lethal to 50% of recipients (and all other subscripter dose levels)
LH	Luteinizing hormone
LDL	Low density liproprotein
LPL	Lipoprotein lipase activity
LOAEL	Lowest-observable-adverse-effect level
LOEL	Lowest-observed-effect level
MCDF	6-Methyl-1,3,8-trichlorodibenzofuran
MFO	Mixed function oxidase
mRNA	Messenger RNA
MNNG	*N*-methyl-*N*-nitrosoguanidine
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NK	Natural killer
NOAEL	No-observable-adverse-effect level
NOEL	No-observed-effect level

LIST OF ABBREVIATIONS (cont.)

OCDD	Octachlorodibenzo-p-dioxin
OCDF	Octachlorodibenzofuran
PAH	Polyaromatic hydrocarbon
PB-Pk	Physiologically based pharmacokinetic
PCB	Polychlorinated biphenyl
OVX	Ovariectomized
PBL	Peripheral blood lymphocytes
PCQ	Quaterphenyl
PeCDD	Pentachlorinated dibenzo-p-dioxin
PeCDF	Pentachlorinated dibenzo-p-dioxin
PEPCK	Phosphopenol pyruvate carboxykinase
PGT	Placental glutathione transferase
PHA	Phytohemagglutinin
PWM	Pokeweed mitogen
ppm	Parts per million
ppq	
ppt	Parts per trillion
RNA	Ribonucleic acid
SAR	Structure-activity relationships
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase

LIST OF ABBREVIATIONS (cont.)

SRBC	Sheep erythrocytes (red blood cells)
$t_{½}$	Half-time
TCAOB	Tetrachloroazoxybenzene
TCB	Tetrachlorobiphenyl
TCDD	Tetrachlorodibenzo-p-dioxin
TEF	Toxic equivalency factors
TGF	Thyroid growth factor
tPA	Tissue plasminogen activator
TNF	Tumor necrosis factor
TNP-LPS	lipopolysaccharide
TSH	Thyroid stimulating hormone
TTR	Transthyretrin
UDPGT	UDP-glucuronosyltransferases
URO-D	Uroporphyrinogen decarboxylase
VLDL	Very low density lipoprotein
v/v	Volume per volume
w/w	Weight by weight

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1. DISPOSITION AND PHARMACOKINETICS

The disposition and pharmacokinetics of 2,3,7,8-TCDD and related compounds have been investigated in several species and under various exposure conditions. There are several reviews on this subject that focus on 2,3,7,8-TCDD and related halogenated aromatic hydrocarbons (Neal et al., 1982; Gasiewicz et al., 1983a; Olson et al., 1983; Birnbaum, 1985). During the last 6 years, considerably more data have been published on this class of compounds that includes 2,3,7,8-substituted CDDs, BDDs, CDFs, BDFs and the coplaner PCBs and PBBs. This chapter reviews the disposition and pharmacokinetics of these agents and identifies congener and species specific factors that may have an impact on the dose-related biological responses of these compounds.

1.1. ABSORPTION/BIOAVAILABILITY FOLLOWING EXPOSURE

The gastrointestinal, dermal and transpulmonary absorption of these compounds are discussed herein because they represent potential routes for human exposure to this class of persistent environmental contaminants. Parenteral absorption is reviewed since this route of exposure has been used to generate disposition and pharmacokinetic data on these compounds.

1.1.1. Oral

1.1.1.1. GASTROINTESTINAL ABSORPTION IN ANIMALS -- A major source of human exposure to 2,3,7,8-TCDD and related compounds is thought to be through the diet. Experimentally, these compounds are commonly administered in the diet or by gavage in an oil vehicle. Gastrointestinal absorption is usually estimated as the difference between the administered dose (100%) and the % of the dose that was not absorbed. The unabsorbed fraction is estimated as the recovery of parent compound in feces within 24-48 hours of a single oral exposure by gavage. Table 1-1 summarizes gastrointestinal absorption data on 2,3,7,8-TCDD and related compounds.

In Sprague-Dawley rats given a single oral dose of 1.0 μg [^{14}C]-2,3,7,8-TCDD/kg bw in acetone:corn oil (1:25, v/v), the fraction absorbed ranged from 66-93% with a mean of 84% (Rose et al., 1976). With repeated oral dosing of rats at 0.1 or 1.0 μg/kg/day (5 days/week for 7 weeks), gastrointestinal absorption

TABLE 1-1

Gastrointestinal Absorption of 2,3,7,8-TCDD and Related Compounds Following a Single Oral Exposure by Gavage

Chemical	Species (Sex)	Dose (μmol/kg)	Dose (μg/kg)	Vehicle	% Administered Dose Absorbed[a] [Mean (Range)]	Reference
CDDs						
2,3,7,8-TCDD	Sprague-Dawley rat (M)	0.16	50	acetone:corn oil (1:7)	70	Piper et al., 1973
2,3,7,8-TCDD	Sprague-Dawley rat (M/F)	0.003	1.0	acetone:corn oil (1:25)	84 (66-93)	Rose et al., 1976
2,3,7,8-TCDD	Hartley guinea pig (F)	0.005	1.45	acetone:corn oil (1:45)	50	Nolan et al., 1979
2,3,7,8-TCDD	Golden Syrian hamster (M)	2.0	650	olive oil	74	Olson et al., 1980
2,3,7,8-TCDD	Human (M)	0.000003	0.001	corn oil	87	Poiger and Schlatter, 1986
1,2,3,7,8-PeCDD	Sprague-Dawley rat (M/F)	0.03	9.2	corn oil	NR (19-71)	Wacker et al., 1986
OCDD	Fischer 344 rat (M)	0.11 1.1 1.1 11	50 500 500 5000	o-dichlorobenzene:Emulphor (1:1) o-dichlorobenzene:corn oil (1:1) corn oil suspension corn oil suspension	12 15 2 5	Birnbaum and Couture, 1988
BDDs						
2,3,7,8-TBDD	Fischer 344 rat (M)	0.001 0.01 0.1 0.5	0.5 5 50 500	Emulphor:ethanol:water (1:1:3)	78 82 60 47	Diliberto et al., 1990
CDFs						
2,3,7,8-TCDF	Fischer 344 rat (M)	0.1 1.0	30.6 306	Emulphor:ethanol (1:1)	90 90	Birnbaum et al., 1980
2,3,7,8-TCDF	Hartley guinea pig (M)	0.02	6	Emulphor:ethanol:water (1:1:8)	90	Decad et al., 1981a
2,3,4,7,8-PeCDF	Fischer 344 rat (M)	0.1 0.5 1.0	34 170 340	corn oil	~70 ~70 ~70	Brewster and Birnbaum, 1987

TABLE 1-1 (cont.)

Chemical	Species (Sex)	Dose		Vehicle	% Administered Dose Absorbed[a] [Mean (Range)]	Reference
		(μmol/kg)	(μg/kg)			
PCBs						
3,3'4,4'-T4CB	C57BL mouse (F)	34.5	10,000	corn oil	77	Wehler et al., 1989

[a]Absorption is generally estimated as the difference between the administered dose (100%) and the % of the dose that was not absorbed. The unabsorbed fraction is estimated as the recovery of parent compound in feces within 48 hours of exposure.

NR = Not reported

of 2,3,7,8-TCDD was observed to be approximately that observed for the single oral exposure (Rose et al., 1976). Oral exposure of Sprague-Dawley rats to a larger dose of 2,3,7,8-TCDD in acetone: corn oil (50 μg/kg) resulted in an average absorption of 70% of the administered dose (Piper et al., 1973).

One study in the guinea pig reported that ~50% of a single oral dose of 2,3,7,8-TCDD in acetone:corn oil was absorbed (Nolan et al., 1979). The gastrointestinal absorption of 2,3,7,8-TCDD was also examined in the hamster, the species most resistant to the acute toxicity of this compound (Olson et al., 1980a). Hamsters were given a single, sublethal, oral dose of [1,6-^{3}H]-2,3,7,8-TCDD in olive oil (650 μg/kg), and an average of 75% of the dose was absorbed. When 2,3,7,8-TCDD was administered to rats in the diet at 7 or 20 ppb (0.5 or 1.4 μg/kg/day) for 42 days, 50-60% of the consumed dose was absorbed (Fries and Marrow, 1975). These findings indicate that oral exposure to 2,3,7,8-TCDD in the diet or in an oil vehicle results in the absorption of >50% of the administered dose.

The intestinal absorption of ^{3}H-2,3,7,8-TCDD has also been investigated in thoracic duct-cannulated rats (Lakshmanan et al., 1986). The investigators concluded that 2,3,7,8-TCDD was absorbed into chylomicrons and transported through the lymphatic system prior to entering the systemic circulation.

The absorption of 2,3,7,8-TBDD in male Fischer 344 rats was studied after oral exposure by gavage at 5 μg/kg in Emulphor:ethanol:water (1:1:3) (Diliberto et al., 1990). The percent of the dose absorbed for this study was defined as 100 -- (% total oral dose in feces on day 1 and 2 -- % total intravenous dose in feces on day 1 and 2) using the intravenous pharmacokinetic data of Kedderis et al. (1991).

The relative absorbed dose or bioavailability of 2,3,7,8-TBDD after oral exposure was estimated at 78, 82, 60 and 47% at dose levels of 0.001, 0.01, 0.1 and 0.5 μmol/kg, respectively. These results suggest nonlinear absorption at the higher doses, with maximal oral absorption at an exposure of ≤0.01 μmol/kg (5 μg/kg).

The absorption of 2,3,7,8-TCDF has been investigated after oral exposure by gavage. Approximately 90% of the administered dose (0.1 and 1.0 μmol/kg) of

2,3,7,8-TCDF in Emulphor:ethanol (1:1) was absorbed in male Fischer 344 rats (Birnbaum et al., 1980). [Emulphor EL-620 is a polyoxyethylated vegetable oil preparation (GAF Corp., New York, NY)]. Similarly, >90% of the administered dose (0.2 μmol/kg, 6 μg/kg and 1-15 μg/kg) of 2,3,7,8-TCDF in Emulphor: ethanol:water (1:1:8) was absorbed in male Hartley guinea pigs (Decad et al., 1981a; Ioannou et al., 1983). Thus, 2,3,7,8-TCDF appears to be almost completely absorbed from the gastrointestinal tract. This may be related to the greater relative solubility of 2,3,7,8-TCDF compared to that of 2,3,7,8-TCDD or 2,3,7,8-TBDD.

The oral bioavailability of 2,3,4,7,8-PeCDF and 3,3',4,4'-TCB in corn oil were similar to that of 2,3,7,8-TCDD (Brewster and Birnbaum, 1987; Wehler et al., 1989; Clarke et al., 1984). Furthermore, 2,3,4,7,8-PeCDF absorption was independent of the dose (0.1, 0.5 or 1.0 μmol/kg). Incomplete and variable absorption of 1,2,3,7,8-PeCDD was reported in rats, with 19-71% of the dose absorbed within the first 2 days after oral exposure (Wacker et al., 1986).

Early studies on the pharmacokinetic behavior of OCDD by Williams et al. (1972) and Norback et al. (1975) demonstrated that OCDD was poorly absorbed after oral exposure. More recently, Birnbaum and Couture (1988) also found that the gastrointestinal absorption of OCDD in rats was very limited, ranging from 2-15% of the administered dose. Lower 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.

1.1.1.2. GASTROINTESTINAL ABSORPTION IN HUMANS -- Poiger and Schlatter (1986) investigated the absorption of 2,3,7,8-TCDD in a 42-year-old man after ingestion of 105 ng ^{3}H-2,3,7,8-TCDD (1.14 ng/kg bw) in 6 mL corn oil and found that >87% of the oral dose was absorbed from the gastrointestinal tract. Following absorption, the half-life for elimination was estimated to be 2120 days.

The above data indicate that gastrointestinal absorption of 2,3,7,8-TCDD and related compounds is variable, incomplete and congener specific. More soluble congeners, such as 2,3,7,8-TCDF, are almost completely absorbed, while the extremely insoluble OCDD is very poorly absorbed. In some cases, absorption has been found to be dose dependent, with increased absorption occurring at lower

doses (2,3,7,8-TBDD, OCDD). The limited data base also suggests that there are no major interspecies differences in the gastrointestinal absorption of these compounds.

1.1.1.3. BIOAVAILABILITY FOLLOWING ORAL EXPOSURE -- Oral exposure of humans to 2,3,7,8-TCDD and related compounds usually occurs as a complex mixture of these contaminants in food, soil, dust, water or other mixtures that would be expected to alter absorption.

The influence of dose and vehicle or adsorbent on gastrointestinal absorption has been investigated in rats by Poiger and Schlatter (1980), using hepatic concentrations 24 hours after dosing as an indicator of the amount absorbed (Table 1-2). Administration of 2,3,7,8-TCDD in an aqueous suspension of soil resulted in a decrease in the hepatic levels of 2,3,7,8-TCDD as compared with hepatic levels resulting from administration of 2,3,7,8-TCDD in 50% ethanol. The extent of the decrease was directly proportional to the length of time the 2,3,7,8-TCDD had been in contact with the soil. When 2,3,7,8-TCDD was mixed in an aqueous suspension of activated carbon, absorption was almost totally eliminated (<0.07% of the dose in hepatic tissues).

Philippi et al. (1981) and Hutter and Philippi (1982) have shown that radiolabeled 2,3,7,8-TCDD becomes progressively more resistant with time to extraction from soil. Similarly, the feeding of fly ash, which contains CDDs, to rats in the diet for 19 days resulted in considerably lower hepatic levels of CDDs than did the feeding of an extract of the fly ash at comparable dietary concentrations of CDDs (van den Berg et al., 1983). The CDDs were tentatively identified as 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 and the difference in hepatic levels noted between fly ash-treated and extract-treated rats was greater for the more highly chlorinated isomers than it was for 2,3,7,8-TCDD. These results indicate the importance of the formulation or vehicle containing the toxin(s) on the relative bioavailability of 2,3,7,8-TCDD, PeCDD and HxCDDs after oral exposure.

Since 2,3,7,8-TCDD in the environment is likely to be absorbed to soil, McConnell et al. (1984) and Lucier et al. (1986) compared the oral bioavailability of 2,3,7,8-TCDD from environmentally contaminated soil to that from

TABLE 1-2

Percentage of 2,3,7,8-TCDD in the Liver of Rats 24 Hours After Oral Administration of 0.5 mL of Various Formulations Containing TCDD*

Formulation	TCDD Dose (ng)	No. of Animals	Percentage of Dose in the Liver
50% ethanol	14.7	7	36.7±1.2
Aqueous suspension of soil (37%, w/w) that had been in contact with TCDD for:			
10-15 hours	12.7, 22.9	17	24.1±4.8
8 days	21.2, 22.7	10	16.0±2.2
Aqueous suspension of activated carbon (25%, w/w)	14.7	6	≤0.07

*Source: Poiger and Schlatter, 1980

w/w = Weight by weight

2,3,7,8-TCDD administered in corn oil in rats and guinea pigs and rats, respectively. As indicated by biological effects and the amount of 2,3,7,8-TCDD in the liver, the intestinal absorption from Times Beach and Minker Stout, Missouri, soil was ~50% less than from corn oil. Shu et al. (1988) reported an oral bioavailability of ~43% in the rat dosed with three environmentally contaminated soil samples from Times Beach, Missouri. This figure did not change significantly over a 500-fold dose range of 2-1450 ng 2,3,7,8-TCDD/kg bw for soil contaminated with ~2, 30 or 600 ppb of 2,3,7,8-TCDD. In studies of other soil types, Umbreit et al. (1986a,b) estimated an oral bioavailability in the rat of 0.5% for soil at a New Jersey manufacturing site and 21% for a Newark salvage yard. These results indicate that bioavailability of 2,3,7,8-TCDD from soil varies between sites and that 2,3,7,8-TCDD content alone may not be indicative of potential human hazard from contaminated environmental materials. Although these data indicate that substantial absorption may occur from contaminated soil, soil type and duration of contact, as suggested from the data that demonstrated decreased extraction efficiency with increasing contact time between soil and 2,3,7,8-TCDD (Philippi et al., 1981; Huetter and Philippi, 1982), may substantially affect the absorption of 2,3,7,8-TCDD from soils obtained from different contaminated sites.

1.1.2. Dermal Absorption. Brewster et al. (1989) examined the dermal absorption of 2,3,7,8-TCDD and three CDFs in male Fischer 344 rats (10 weeks old; 200-250 g). The fur was clipped from the intrascapular region of the back of each animal. A single compound was then applied over a 1.8 cm^2 area of skin in 60 μL of acetone and covered with a perforated stainless steel cap. Table 1-3 summarizes data on the absorption of each compound at 3 days after a single dermal exposure. At an exposure of 0.1 μmol/kg, the absorption of 2,3,7,8-TCDF (49% of administered dose) was greater than that of 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDF and 2,3,7,8-TCDD. For each compound, the relative absorption (percentage of administered dose) decreased with increasing dose while the absolute absorption (μg/kg) increased nonlinearly with dose. Results also suggest that the majority of the compound remaining at the skin exposure site was associated with the stratum corneum and did not penetrate through to the dermis. In a subsequent

TABLE 1-3

Dermal Absorption of 2,3,7,8-TCDD and Related Compounds in the Rat[a]

Chemical	Dose		% Administered Dose	
	(μmol/kg)	(μg/kg)	Skin Site[b]	Absorbed
2,3,7,8-TCDD	0.00015	0.05	61.73±4.37	38.27±4.37
	0.001	0.32	59.71±1.90	40.29±1.89
	0.01	3.2	72.60±0.41	27.40±0.41
	0.1	32	82.21±2.85	17.78±2.85
	0.5	160	80.92±2.74	19.08±2.74
	1.0	321	82.68±3.69	17.30±3.67
2,3,7,8-TCDF	0.1	31	51.18±11.95	48.84±11.95
	0.5	153	82.14±11.22	17.86±11.22
	1.0	306	88.70±5.17	11.32±5.17
1,2,3,7,8-PeCDF	0.1	34	74.72±3.58	25.27±3.58
	0.5	170	91.67±2.46	8.33±2.46
	1.0	340	84.23±5.44	15.76±5.44
2,3,4,7,8-PeCDF	0.1	34	65.77±4.80	34.19±4.78
	0.5	170	75.50±1.81	24.50±1.80
	1.0	340	81.84±1.67	18.16±1.67

[a]Source: Brewster et al., 1989

[b]Values are the mean±SD of three to four animals and represent the amount of administered dose of radiolabeled congener remaining at the application site 3 days after dermal exposure.

study, Banks and Birnbaum (1991a) examined the rate of absorption of 2,3,7,8-TCDD over 120 hours after the dermal application of 200 pmol (1 nmol/kg) to male Fischer 344 rats. The absorption kinetics appeared to be first-order, with an absorption rate constant of 0.005 $hour^{-1}$. Using a similar exposure protocol, the dermal absorption of 2,3,7,8-TCDF was found to follow a first-order process with a rate constant of 0.009 $hour^{-1}$ (Banks and Birnbaum, 1991b). Together, these results on dermal absorption indicate that at lower doses (≤0.1 μmol/kg), a greater percent of this administered dose of 2,3,7,8-TCDD and three CDFs was absorbed. Nonetheless, the rate of absorption of 2,3,7,8-TCDD is still very slow (rate constant of 0.005 $hour^{-1}$) even following a low dose dermal application of 200 pmol (1 nmol/kg). Results from Table 1-3 also suggest that the dermal absorption of 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF and 1,2,3,7,8-PeCDF occurs at a very slow rate. Using a similar exposure protocol, the dermal absorption of 2,3,7,8-TBDD was only 30-40% of that observed for 2,3,7,8-TCDD (Jackson et al., 1991).

Rahman et al. (1992) and Gallo et al. (1992) compared the *in vitro* permeation of 2,3,7,8-TCDD through hairless mouse and human skin. In both species, the amount of 2,3,7,8-TCDD permeated increased with the dose, but the percent of the dose permeated decreased with increasing dose. The permeability coefficient of 2,3,7,8-TCDD in human skin was about one order of magnitude lower than that in mouse skin. The hairless mouse skin does not appear to be a suitable model for the permeation of 2,3,7,8-TCDD through human skin since the viable tissues were the major barrier to 2,3,7,8-TCDD permeation in hairless mouse skin, while the stratum corneum layer provided the greater resistance in human skin. A significant increase in 2,3,7,8-TCDD permeation through human skin was observed when the skin was damaged by tape-stripping. Gallo et al. (1992) suggested that washing and/or tape-stripping of the exposed area might remove most of the 2,3,7,8-TCDD and reduce the potential for systemic exposure and toxicity since most of the 2,3,7,8-TCDD remained within the horny layer of human skin even at 24 hours following exposure.

Weber et al. (1991) also investigated the penetration of 2,3,7,8-TCDD into human cadaver skin at concentrations of 65-6.5 ng/cm^2. This study also found that the stratum corneum acted as a protective barrier, as its removal increased

the amount of 2,3,7,8-TCDD absorbed into layers of the skin. With intact skin and acetone as the vehicle, the rate of penetration of 2,3,7,8-TCDD into the dermis ranged from 6-170 pg/hour/cm^2, while penetration into the dermis and epidermis ranged from 100-800 pg/hour/cm^2. With mineral oil as the vehicle, there was about a 5- to 10-fold reduction in the rate of penetration of 2,3,7,8-TCDD into the intact skin.

1.1.2.1. BIOAVAILABILITY FOLLOWING DERMAL EXPOSURE -- Dermal exposure of human to 2,3,7,8-TCDD and related compounds usually occurs as a complex mixture of these contaminants in soil, oils or other mixtures which would be expected to alter absorption. Poiger and Schlatter (1980) presented evidence that the presence of soil or lipophilic agents dramatically reduces dermal absorption of 2,3,7,8-TCDD compared to absorption of pure compound dissolved in solvents. In a control experiment, 26 ng of 2,3,7,8-TCDD in 50 μL methanol was administered to the skin of rats, and 24 hours later the liver contained 14.8±2.6% of the dose. By comparing this value to the hepatic levels obtained after oral administration in 50% ethanol (in the same study), the amount absorbed from a dermal application can be estimated at ~40% of the amount absorbed from an equivalent oral dose. This comparison assumes that hepatic levels are valid estimates of the amount absorbed from both oral and dermal routes and that absorption from methanol is equivalent to absorption from 50% ethanol. The dose-dependent distribution of 2,3,7,8-TCDD in the liver is another factor that may limit quantitative conclusions regarding bioavailability which are based solely on hepatic levels following exposure to 2,3,7,8-TCDD. As compared with dermal application in methanol, dermal application of 2,3,7,8-TCDD to rats in vaseline or polyethylene glycol reduced the percentage of the dose in hepatic tissue to 1.4 and 9.3%, respectively, but had no observable effect on the dose of 2,3,7,8-TCDD required to induce skin lesions (~1 μg/ear) in the rabbit ear assay. Application of 2,3,7,8-TCDD in a soil/water paste decreased hepatic 2,3,7,8-TCDD to ~2% of the administered dose and increased the amount required to produce skin lesions to 2-3 μg in rats and rabbits, respectively. Application in an activated carbon/water paste essentially eliminated absorption, as measured by percent of dose in the liver, and increased the amount of 2,3,7,8-TCDD required to produce

skin lesions to ~160 μg. These results suggest that the dermal absorption and acnegenic potency of 2,3,7,8-TCDD depend on the formulation (vehicle or adsorbent) containing the toxin.

Shu et al. (1988) investigated the dermal absorption of soil-bound 2,3,7,8-TCDD in rats. Relative dermal bioavailability was estimated by comparing the level of 2,3,7,8-TCDD in the liver of rats given soil-bound 2,3,7,8-TCDD dermally to that of rats given oral doses of 2,3,7,8-TCDD dissolved in corn oil. The level of 2,3,7,8-TCDD in livers of rats dosed orally with 2,3,7,8-TCDD in corn oil, following correction for unabsorbed 2,3,7,8-TCDD, is assumed to represent 100% bioavailability. The dermal penetration of 2,3,7,8-TCDD after 4 hours of contact with skin was ~60% of that after 24 hours of contact. After 24 hours of contact with the skin, the degree of dermal uptake from contaminated soil was ~1% of the administered dose. The authors observed that the degree of uptake does not appear to be influenced significantly by the concentration of 2,3,7,8-TCDD in soil, the presence of crankcase oil as co-contaminants or by environmentally- versus laboratory-contaminated soil.

A major limitation of the above studies is the uncertainty regarding the extrapolation of dermal absorption data on these compounds from the rat to the human. The *in vitro* dermal uptake of 2,3,7,8-TCDD has been investigated in hairless mouse and human skin (Gallo et al., 1992; Rahman et al., 1992). *In vitro* dermal uptake of 2,3,7,8-TCDD from laboratory-contaminated soil found that aging of soils (up to 4 weeks) and the presence of additives (2,4,5-trichlorophenol and motor oil) in the soil did not have any significant effect of dermal uptake (Gallo et al., 1992). Since most of the 2,3,7,8-TCDD remained in the stratum corneum layer of human skin, the permeation of 2,3,7,8-TCDD was significantly lower in human than in hairless mouse skin. Although there are no published quantitative *in vivo* data on the dermal absorption of 2,3,7,8-TCDD and related compounds in the human, there are very limited data on the rhesus monkey. Brewster et al. (1988) found that 1,2,3,7,8-PeCDF was poorly absorbed in the monkey after dermal application with <1% of the administered dose being absorbed in 6 hours. This provides further evidence for the very slow rate of dermal absorption of 2,3,7,8-TCDD and related compounds.

1.1.3. Transpulmonary Absorption. The use of incineration as a means of solid and hazardous waste management results in the emission of contaminated particles that may contain TCDD and related compounds into the environment. Thus, significant exposure to TCDD and related compounds may result from inhalation of contaminated fly ash, dust and soil. In an attempt to address the bioavailability and potential health implications of inhaling contaminated particles, Nessel et al. (1990) examined the potential for transpulmonary absorption of TCDD after intratracheal instillation of the compound administered to female Sprague-Dawley rats either in a corn oil vehicle or as a laboratory-prepared contaminant of gallium oxide particles. Several biomarkers of systemic absorption were measured, including the dose-dependent effects of TCDD on hepatic microsomal cytochrome P-450 content, AHH activity and liver histopathology. Significant dose-related effects were observed at an exposure of ≥0.55 μg TCDD/kg. The authors found that induction was slightly higher when animals received TCDD in corn oil than when animals received TCDD-contaminated particles and was comparable to induction after oral exposure. The results from Nessel et al. (1990) indicate that systemic effects occur after pulmonary exposure to TCDD, suggesting that transpulmonary absorption of TCDD does occur.

The transpulmonary absorption of 2,3,7,8-TCDD was assessed in male Fischer 344 rats following intratracheal instillation of a 1 nmol/kg dose in Emulphor:ethanol:water (Diliberto et al., 1992). Transpulmonary absorption was ~92%, suggesting that there was almost complete absorption of 2,3,7,8-TCDD by inhalation under these conditions. Similar results were also observed for the transpulmonary absorption of 2,3,7,8-TBDD under similar exposure conditions (Diliberto et al., 1991). These results suggest that the transpulmonary absorption of 2,3,7,8-TCDD and 2,3,7,8-TBDD was similar to that observed following oral exposure.

1.1.4. Parenteral Absorption. In an effort to obtain more reproducible and complete absorption of 2,3,7,8-TCDD and related compounds for pharmacokinetic studies, Abraham et al. (1989) investigated the absorption of 2,3,7,8-TCDD after parenteral application in rats, using various vehicles. These investigators observed optimal results with the subcutaneous injection of 2,3,7,8-TCDD using a mixture of toluene:DMSO (1:2) as vehicle. At 3 and 5 days after treatment, the

percentages of administered dose remaining at the injection site under the skin of the back were ~10 and 2%, respectively. The vehicle did not cause adverse effects at an applied volume of 0.2 mL/kg bw. The absorption of a defined mixture of CDDs and CDFs in the rat was also examined after subcutaneous injection using toluene:DMSO (1:2) as a vehicle. Of the 97 congeners analyzed, 70 were ≥95% absorbed 7 days after exposure, 21 were 90-95% absorbed and 1,2,3,9-TCDD, 1,2,3,6,7,9-/ 1,2,3,6,8,9-HxCDD, 1,2,3,4,6,7,9-HpCDD, OCDD, 1,2,4,6,8,9-HxCDF and 1,2,3,7,8,9-HxCDF were 84-89% absorbed. Greater than 90% absorption of CDDs and CDFs was also observed under these conditions in the marmoset monkey, with the exception of 1,2,3,4,7,8,9-HpCDF, OCDF and OCDD, which had ~50-80% of the administered dose absorbed (Neubert et al., 1990; Abraham et al., 1989). Although the absorption of CDDs and CDFs after subcutaneous administration in toluene:DMSO (1:2) is somewhat slow, in rats and monkeys, absorption of most congeners was >90% within 7 days. Even for highly chlorinated insoluble congeners, such as OCDD and OCDF, subcutaneous absorption was >84% in the rat and >50% in the monkey.

Less complete and slower absorption of CDDs and CDFs was observed after subcutaneous injection of these compounds using an oil-containing vehicle (Brunner et al., 1989; Abraham et al., 1989). Using a corn oil:acetone vehicle (24:1, v/v), Lakshmanan et al. (1986) observed that only 7% of the administered dose of 2,3,7,8-TCDD was absorbed 24 hours after subcutaneous injection and that only 35% was absorbed after intraperitoneal injection. Also, Brunner et al. (1989) reported that intraperitoneal administration of CDDs and CDFs revealed a delayed absorption from the abdominal cavity which varied for the different congeners. Therefore, concentrations measured in abdominal adipose tissue after intraperitoneal administration may not represent average values of adipose tissue in the whole body, particularly at early time points following exposure.

1.2. DISTRIBUTION

1.2.1. Distribution in Blood and Lymph. Once a compound is absorbed, its distribution is regulated initially by its binding to components in blood and its ability to diffuse through blood vessels and tissue membranes. Lakshmanan et al. (1986) investigated the absorption and distribution of 2,3,7,8-TCDD in thoracic duct-cannulated rats. Their results suggest that following gastrointestinal

absorption, 2,3,7,8-TCDD is absorbed primarily by the lymphatic route and is transported predominantly by chylomicrons. Ninety percent of the 2,3,7,8-TCDD in lymph was associated with the chylomicron fraction. The plasma disappearance of 2,3,7,8-TCDD-labeled chylomicrons followed first-order decay kinetics, with 67% of the compound leaving the blood compartment very rapidly ($t_{1/2}$=0.81 minutes), whereas the remainder of the 2,3,7,8-TCDD had a $t_{1/2}$ of 30 minutes. 2,3,7,8-TCDD was then found to distribute primarily to the adipose tissue and the liver.

In vitro studies have investigated the distribution of 2,3,7,8-TCDD in human whole blood. Henderson and Patterson (1988) found ~80% of the compound associated with the lipoprotein fraction, 15% associated with protein (primarily human serum albumin) and 5% associated with cellular components. Theoretical and limited experimental data also suggest that 2,3,7,8-TCDD and related compounds may be associated with plasma prealbumin (McKinney et al., 1985; Pedersen et al., 1986). The distribution of [^{3}H]-2,3,7,8-TCDD among lipoprotein fractions from three fasting, normolipemic donors indicated a greater percentage associated with LDL (55.3±9.03% SD) than with VLDL (17.4±9.07% SD) or HDL (27.3±10.08% SD). The distribution of 2,3,7,8-TCDD among the lipoprotein fractions was similar to that reported earlier by Marinovich et al. (1983). When the binding of 2,3,7,8-TCDD was calculated per mole of lipoprotein, it was suggested that the maximal binding capacity was exerted by VLDL, followed by LDL and HDL (Marinovich et al., 1983). The results also suggest that variations in the amounts of each lipoprotein class may alter the distribution of 2,3,7,8-TCDD among lipoproteins in a given subject. Significant species differences also exist; in the case of the rat, which has markedly lower plasma lipids compared to humans, 2,3,7,8-TCDD was distributed almost equally among the lipoprotein fractions (Marinovich et al., 1983).

In addition, there is indirect evidence that suggests that the binding of 2,3,7,8-TCDD to lipoproteins may alter the pharmacokinetics and toxic potency of the compound. Marinovich et al. (1983) found that experimentally induced hyperlipidemia in rats delayed the development of overt toxicity (lethality). However, the disposition of 2,3,7,8-TCDD was not investigated under these conditions. These investigators suggest that the release of lipoprotein-bound

2,3,7,8-TCDD is related to the metabolic turnover of lipoproteins. In hyperlipidemic rats, the turnover of VLDL and LDL is delayed significantly compared to normolipidemic animals, and this may contribute to the plasma lipoprotein binding modifying the toxicity of 2,3,7,8-TCDD in hyperlipidemic rats.

The uptake of lipoprotein-associated 2,3,7,8-TCDD by cultured human fibroblasts found the time- and temperature-dependent cellular uptake was greatest from LDL, intermediate from HDL and least from serum (Shireman and Wei, 1986). Decreased cellular uptake of LDL and 2,3,7,8-TCDD was observed in mutant fibroblasts, which lack the normal cell membrane receptor for LDL. This provides some evidence that specific binding of LDL and the LDL receptor pathway may account for some of the rapid early uptake of 2,3,7,8-TCDD with LDL entry. The results suggest that the entry of 2,3,7,8-TCDD into cells may not be solely by simple diffusion. However, nonspecific binding of the LDL and transfer of 2,3,7,8-TCDD from LDL to the cell membranes are probably also important, since significant time- and temperature-dependent uptake of 2,3,7,8-TCDD and LDL occurred in the mutant fibroblasts.

Thus, upon absorption, 2,3,7,8-TCDD and probably related compounds are bound to chylomicrons, lipoproteins and other serum proteins that assist in distributing these uncharged, lipophilic compounds throughout the vascular system. These compounds then partition from blood components into cellular membranes and tissues, probably largely by passive diffusion. In addition, cellular uptake may be facilitated partly through the cell membrane LDL receptor, the hepatic receptor for albumin (Weisiger et al., 1981) and/or other systems.

1.2.2. Tissue Distribution. Once absorbed into blood, 2,3,7,8-TCDD and related compounds readily distribute to all organs. Tissue distribution within the first hour after exposure parallels blood levels and reflects physiological parameters such as blood flow to a given tissue and relative tissue size. For example, high initial concentrations of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDF and 3,3',4,4'-TCB were observed in highly perfused tissue such as the adrenal glands during the 24-hour period after a single exposure (Birnbaum et al., 1980; Olson et al., 1980; Pohjanvirta et al., 1990; Brewster and Birnbaum, 1988; Durham and Brouwer, 1990). A high percentage of the dose of 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF was also found in muscle within the first hour after intravenous exposure, due to the large

volume of this tissue (Birnbaum et al., 1980; Birnbaum, 1985; Brewster and Birnbaum, 1988). Nevertheless, within several hours the liver, adipose tissue and skin become the primary sites of disposition, when expressed as percent of administered dose per g tissue and as percent of dose per organ. Liver, adipose tissue, skin and thyroid were the only tissue to show an increase in the concentration of 2,3,7,8-TCDD during the initial 4 days after a single intraperitoneal exposure of rats (Pohjanvirta et al., 1990). In this study, a similar general pattern of disposition was observed in Han/Wistar and Long-Evans rats which are respectively most resistant and susceptible to the acute toxicity of 2,3,7,8-TCDD (Pohjanvirta et al., 1990).

Table 1-4 illustrates the tissue distribution of 2,3,7,8-TCDD in female Wistar rats 7 days after a single subcutaneous exposure (Abraham et al., 1988). This general pattern of distribution is similar to that observed in mice, rats, rhesus monkeys, hamsters and guinea pigs, where liver and adipose tissue consistently have the highest concentrations of 2,3,7,8-TCDD (Piper et al., 1973; Fries and Marrow, 1975; Rose et al., 1976; Allen et al., 1975; Van Miller et al., 1976; Kociba et al., 1978a,b; Gasiewicz et al., 1983; Manara et al., 1982; Olson et al., 1980; Gasiewicz and Neal, 1979; Birnbaum, 1986; Pohjanvirta et al., 1990; Abraham et al., 1988). A similar pattern of disposition also was observed for 2,3,7,8-TCDF in the guinea pig, rat, C57BL/6J and DBA/2J mouse and rhesus monkey, with 2,3,7,8-TCDF concentrations highest in liver and adipose tissue (Decad et al., 1981b; Birnbaum et al., 1980, 1981). In summary, there do not appear to be major species or strain differences in the tissue distribution of 2,3,7,8-TCDD and 2,3,7,8-TCDF, with the liver and adipose tissue being the primary disposition sites.

The tissue distribution of the coplanar PCBs and PBBs also appears to be similar to that of 2,3,7,8-TCDD and 2,3,7,8-TCDF. Limited studies in rats and mice found that 3,3',4,4'-TCB, 3,3',4,4'-TBB and 3,3'4,4'5,5'-HxBB distributed preferentially to adipose tissue and liver (Clarke et al., 1983, 1984; Millis et al., 1985; Wehler et al., 1989; Clevenger et al., 1989).

While the liver and adipose tissue contain the highest concentrations of 2,3,7,8-TCDD and 2,3,7,8-TCDF, there are some congener-specific differences in the relative tissue distribution of related compounds. 2,3,7,8-TBDD and

TABLE 1-4

Tissue Distribution of [^{14}C]-2-3,7,8-TCDD in Female Wistar Rats[a,b]

Tissue	Range of 2,3,7,8-TCDD Concentrations (ng/g)
Liver	29.23-30.99
Adipose tissue	3.72-4.14
Adrenal glands	0.89-1.08
Ovaries	0.76-0.96
Thymus	0.60-1.05
Skin	0.64-0.68
Lung	0.32-0.33
Kidney	0.27-0.29
Pancreas	0.21-0.31
Spleen	0.18-0.23
Serum	0.16-0.18
Bone (with marrow)	0.16-0.16
Muscle	0.08-0.12
Brain	0.07-0.09

[a]Source: Abraham et al., 1988

[b]Distribution was assessed 7 days after a single subcutaneous exposure (3 μg/kg bw)

1,2,3,7,8-PeCDD disposition in the rat was very similar to that of 2,3,7,8-TCDD (Kedderis et al., 1991; Wacker et al., 1986). The hepatic concentration of OCDD and 2,3,4,7,8-PeCDF in the rat, however, was approximately 10- to 20-fold greater than that in adipose tissue, which generally contains the second highest levels of these compounds (Birnbaum and Couture, 1988; Norback et al., 1975; Williams et al., 1972; Brewster and Birnbaum, 1987). The tissue distribution of a defined mixture of CDDs and CDFs (28.8 μg CDDs+CDFs/kg bw containing 120 ng 2,3,7,8-TCDD/kg bw) was measured in marmoset monkeys 7 days after a single subcutaneous exposure (Abraham et al., 1990). For most of the 2,3,7,8-substituted congeners, the highest concentrations were detected in hepatic and adipose tissue, with correspondingly lower values detected in kidney, brain, lung, heart, thymus or testes. The hepatic and adipose tissue concentrations were similar for 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,7,8-TCDF and 1,2,3,7,8-/ 1,2,3,4,8-PeCDF. Nonetheless, the hepatic concentrations were approximately 10-fold or more greater than those of adipose tissue for 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-/1,2,3,4,7,9-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF and OCDF. The lungs and thymus contained higher concentrations of all of the above congeners than were detected in kidney, brain, heart and testes. Unexpectedly, the concentrations of the above HxCDDs, HpCDDs, OCDD and HxCDFs were similar in the adipose tissue, lungs and thymus. In the case of HpCDFs and OCDF, the concentrations were greater in the lungs than the adipose tissue. The enhanced disposition of highly chlorinated congeners to the lungs and thymus is of interest and deserves further investigation. For example, it is possible that the high concentration in the lungs could be related to the insolubility of these compounds.

Whole-body autoradiography of mice and rats after intravenous administration of [^{14}C]-2,3,7,8-TCDD showed a selective localization of radioactivity in the liver and nasal olfactory mucosa (Appelgren et al., 1983; Gillner et al., 1987). The selective localization of 2,3,7,8-TCDD in the nasal olfactory mucosa was apparently overlooked by other distribution studies that only examined selected organs. Gillner et al. (1987) found no 2,3,7,8-TCDD-derived radioactivity in the

olfactory mucosa after solvent extraction of sections, suggesting that 2,3,7,8-TCDD was not covalently bound in this tissue. In addition, Gillner et al. (1987) reported induction of ^{m}RNA coding for cytochrome P-4501A2 in the absence of P-4501A1 induction in olfactory mucosa of rats. The selective distribution of 2,3,7,8-TCDD in the liver and olfactory mucosa correlates with the tissue specific induction of cytochrome P-4501A2, which represents a potential sequestration (binding) protein (see Section 1.2.5). Increases in the incidence of squamous cell carcinoma of nasal turbinates and carcinoma of the liver were observed in rats after a 2-year exposure to 2,3,7,8-TCDD in rat chow (Kociba et al., 1978); however, this effect was not observed in nasal tissues of mice or rats intubated with 2,3,7,8-TCDD. Gillner et al. (1987) suggested that 2,3,7,8-TCDD may not be an initiator in this tissue and indicated that future studies should investigate the possibility that 2,3,7,8-TCDD may act as a promoter or cocarcinogen in nasal tissue.

Evidence has also been reported that suggests that 2,3,7,8-TCDD uptake and retention by the liver is dependent on the cell type within the liver. Hakansson et al. (1989) found that at 4 days after exposure of rats to 2,3,7,8-TCDD, 60% of the dose distributed to hepatocytes and 12% was retained by stellate cells. Half-lives for 2,3,7,8-TCDD in hepatocytes and stellate cells were also calculated to be 13 and 50 days, respectively, suggesting that 2,3,7,8-TCDD is more persistent in nonparenchymal cells. Further studies are needed to understand the pharmacokinetic and pharmacodynamic significance of the cell-specific distribution of 2,3,7,8-TCDD and related compounds.

1.2.2.1. TISSUE DISTRIBUTION IN HUMANS -- Fachetti et al. (1980) reported tissue concentrations of 2,3,7,8-TCDD at levels of 1-2 ng/g in adipose tissue and pancreas, 0.1-0.2 ng/g in the liver and ≤0.1 ng/g in thyroid, brain, lung, kidney and blood in a woman who died 7 months after potential exposure to 2,3,7,8-TCDD from the Seveso accident. This pattern of 2,3,7,8-TCDD distribution, however, may not be representative for humans since the woman at the time of death had an adenocarcinoma (which was not considered related to the accident) involving the pancreas, liver and lung.

Ryan et al. (1985a) examined the distribution of 2,3,7,8-TCDD in two humans at autopsy. They determined on a weight basis that 2,3,7,8-TCDD distributed in descending order to fat (~6 ppt) and liver (~2 ppt), with levels in muscle and kidney below detection; however, 2,3,7,8-TCDD levels compared on a per lipid basis were similar between tissues. These data should be interpreted with caution, since only two subjects were examined and one of the subjects was suffering from fatty liver syndrome; therefore, the data cannot be generalized to the entire population.

Poiger and Schlatter (1986) estimated that ~90% of the body burden of 2,3,7,8-TCDD was sequestered in the fat after a volunteer ingested ^{3}H-2,3,7,8-TCDD in corn oil at a dose of 1.14 ng/kg. During this 135-day study, elevated radioactivity was detected in the blood only during the first 2 days after treatment. The data would be consistent with the high lipid bioconcentration potential of 2,3,7,8-TCDD in humans, as calculated by Geyer et al. (1986) from daily intake assumptions, levels in human adipose tissue and pharmacokinetic models. Geyer et al. (1986) estimated a BCF of between 104 and 206 for 2,3,7,8-TCDD in human adipose tissue.

In human adipose tissue, levels of 2,3,7,8-TCDD averaging 5-10 ppt have been reported for background populations in St. Louis, MO, by Graham et al. (1986), in Atlanta, GA, and Utah by Patterson et al. (1986), and in Canada by Ryan et al. (1985b). Sielken (1987) evaluated these data and concluded that the levels of 2,3,7,8-TCDD in human adipose are log-normally distributed and positively correlated with age. Among the observed U.S. background levels of 2,3,7,8-TCDD in human adipose tissue, more than 10% were >12 ppt.

Patterson et al. (1987) developed a HRGC/HRMS analysis for 2,3,7,8-TCDD in human serum. The arithmetic mean of the individual human serum samples was 47.9 ppq on a whole-weight basis and 7.6 ppt on a lipid-weight basis. Paired human serum and adipose tissue levels of 2,3,7,8-TCDD have been compared by Patterson et al. (1988) and Kahn et al. (1988). Both laboratories reported a high correlation between adipose tissue and serum 2,3,7,8-TCDD levels when the samples were adjusted for total lipid content. This correlation indicates that serum

2,3,7,8-TCDD is a valid estimate of the 2,3,7,8-TCDD concentration in adipose tissue.

In a study of potentially heavily exposed Vietnam veterans, MMMR (1988) reviewed an Air Force study of Ranch Hand veterans who were either herbicide loaders or herbicide specialists in Vietnam. The mean serum 2,3,7,8-TCDD levels of 147 Ranch Hand personnel was 49 ppt in 1987, based on total lipid-weight, while the mean serum level of the 49 controls was 5 ppt. In addition, 79% of the Ranch Hand personnel and 2% of the controls had 2,3,7,8-TCDD levels ≥10 ppt. The distribution of 2,3,7,8-TCDD levels in this phase of the Air Force health study indicates that only a small number of Ranch Hand personnel had unusually heavy 2,3,7,8-TCDD exposure. This report also estimated the half-life of 2,3,7,8-TCDD in humans to be ~7 years on the basis of 2,3,7,8-TCDD levels in serum samples taken in 1982 and 1987 from 36 of the Ranch Hand personnel who had 2,3,7,8-TCDD levels >10 ppt in 1987. Similar results were obtained by Kahn et al. (1988) who compared 2,3,7,8-TCDD levels in blood and adipose tissue of Agent Orange-exposed Vietnam veterans and matched controls (Kahn et al., 1988). This study also examined moderately exposed Vietnam veterans who handled herbicides regularly while in Vietnam. Although this study can distinguish moderately exposed men from others, the data do not address the question of identifying persons whose exposures are relatively low and who constitute the bulk of the population, both military and civilian, who may have been exposed to greater than background levels of 2,3,7,8-TCDD.

1.2.3. Time-Dependent Tissue Distribution. 2,3,7,8-TCDD and related compounds exhibit congener specific disposition, which depends on tissue, species and time after a given exposure. In general, these compounds are cleared rapidly from the blood and distributed to liver, muscle, skin, adipose tissue and other tissues within the first hour(s) after exposure. This is followed by redistribution to the liver and adipose tissue, which exhibit increasing tissue concentrations over several days after exposure. Elimination from tissues then occurs at rates that are congener-, tissue- and species-specific. Thus, the ratio of the concentration of 2,3,7,8-TCDD and related compounds in different tissues (i.e., liver/adipose) may not remain constant over an extended period after a single exposure. Abraham et al. (1988) examined the concentrations of 2,3,7,8-TCDD in liver and

adipose tissue of female Wistar rats over a 91-day period after a single subcutaneous exposure at a dose of 300 ng/kg bw (Figure 1-1). The maximum concentration of 2,3,7,8-TCDD in the liver and adipose tissue was reached at 3 and 7 days after exposure, respectively. The liver/adipose tissue concentration ratio does not remain constant over time since the concentration of 2,3,7,8-TCDD decreases more rapidly in the liver than in the adipose tissue. For example, the liver/adipose tissue concentration ratio (for 2,3,7,8-TCDD) was 10.3 at 1 day after exposure and 0.5 at 91 days after exposure (Figure 1-1). Results from other disposition studies also indicate that the ratio of the concentration of 2,3,7,8-TCDD and related compounds in liver, adipose tissue and other tissues does not remain constant over an extended period after a single exposure (Pohjanvirta et al., 1990; Birnbaum, 1986; Birnbaum et al., 1980; Decad et al., 1981a; Birnbaum and Couture, 1988; Olson et al., 1980; Kedderis et al., 1991; Brewster and Birnbaum, 1987, 1988; Neubert et al., 1990). This relationship is important in attempting to correlate dose-response data with tissue concentrations of 2,3,7,8-TCDD and related compounds.

In an attempt to maintain constant 2,3,7,8-TCDD levels in tissues to study long-term effects, Krowke et al. (1989) investigated several loading-dose/maintenance-dose exposure regimens. They found that similar liver/adipose tissue concentrations ranging from 5-8 could be maintained in rats over a 22-week period using a loading dose of 25 μg/kg followed by weekly maintenance doses of 5 μg/kg.

A large body of data on the tissue concentrations of 2,3,7,8-TCDD and related compounds over time after exposure can be evaluated by estimating congener-specific half-life values for a given tissue and species. Table 1-5 summarizes pharmacokinetic elimination parameters for 2,3,7,8-TCDD and related compounds from major tissue depots. Data from Abraham et al. (1988) (see Figure 1-1) were used to estimate the half-life for 2,3,7,8-TCDD in the liver and adipose tissue of rats (Table 1-5). The decrease in the 2,3,7,8-TCDD concentration in adipose tissue is a linear function in the semi-logarithmic plot in Figure 1-1 (log concentration versus time), which indicates apparent first-order elimination kinetics with a half-life of 24.5 days (Table 1-5). Liver tissue exhibits a biphasic (two-component) exponential decay pattern with a half-life of 11.5 days for the first component (days 10-49) and a half-life of 16.9 days

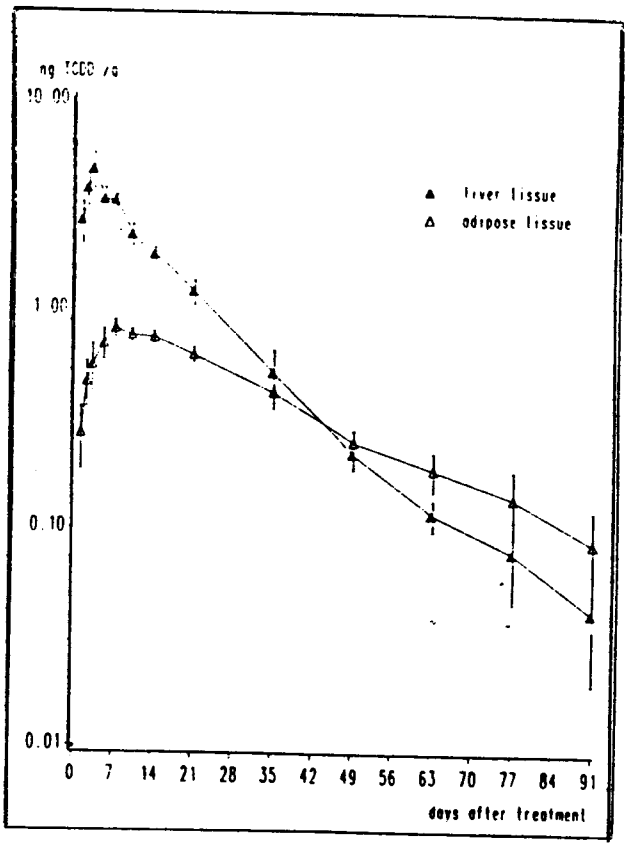

FIGURE 1-1

Time Course of the Concentration of ^{14}C-TCDD in Rat Liver and Adipose Tissue After a Single Subcutaneous Injection of 300 ng TCDD/kg bw to Female Rats (M±SD).

Source: Abraham et al., 1988

TABLE 1-5

Elimination of 2,3,7,8-TCDD and Related Compounds from Major Tissue Depots

Chemical	Species (Sex)	Dose	Tissue	Half-Life (days)	Remarks	Reference
CDDs						
2,3,7,8-TCDD	Wistar rat (F)	0.3 µg/kg, s.c.	liver liver liver adipose	11.5 16.9 13.6 24.5	95% Confidence interval (time period investigated): 10.7-12.3 (10-49 days) 14.0-21.4 (49-91 days) 12.8-14.4 (10-91 days) 22.4-26.8 (14-91 days)	Abraham et al., 1988
2,3,7,8-TCDD	Wistar rat (M)	1.0 µg//kg, i.p.	liver adipose	37.1 53.2	Tissue levels were measured for 20 weeks following exposure	Lakshmanan et al., 1986
2,3,7,8-TCDD	Sprague-Dawley rat (M)	7 or 20 ppb in diet for 42 days	liver	11	85% total dose	Fries and Marrow, 1975
2,3,7,8-TCDD	Sprague-Dawley rat (F)	7 or 20 ppb in diet for 42 days	liver	13	70% of total dose	Fries and Marrow, 1975
2,3,7,8-TCDD	C57BL/6J mice (M) Ah^b/Ah^d	0.5 µg/kg, i.p.	liver adipose skin	8.5 10.3 16.0	Pool size (% of total dose): 36.8 23.6 7.6	Birnbaum, 1986
2,3,7,8-TCDD	C57BL/6J mice (M) Ah^d/Ah^d	0.5 µg/kg, i.p.	liver adipose skin	7.1 7.6 14.9	Pool size (% of total dose): 20.6 31.3 10.2	Birnbaum, 1986
2,3,7,8-TCDD	DBA/2J mice (F) Ah^b/Ah^d	0.5 µg/kg, i.p.	liver adipose skin	12.4 13.3 13.2	Pool size (% of total dose): 29.2 30.9 21.4	Birnbaum, 1986
2,3,7,8-TCDD	DBA/2J mice (F) Ah^d/Ah^d	0.5 µg/kg, i.p.	liver adipose skin	11.9 11.8 12.8	Pool size (% of total dose): 20.2 42.3 26.6	Birnbaum, 1986
2,3,7,8-TCDD	rhesus monkey (F)	25 ppt in diet	adipose	391±88	Mean±SE (n=7)	Bowman et al., 1989

TABLE 1-5 (cont.)

Chemical	Species (Sex)	Dose	Tissue	Half-Life (days)	Remarks	Reference
OCDD	Fischer 344 rat (M)	50 µg/kg, i.v.	liver adipose skin	84 38 3 69	Pool size (% of total dose): 72.7 7.1 9.0, 1st component 0.3, 2nd component	Birnbaum and Couture, 1988
BDDs						
2,3,7,8-TBDD	Fischer 344 rat (M)	0.5 µg/kg, i.v. (0.001 µmol/kg)	liver adipose skin muscle blood	4.5 16.5 57.8 2.5 57.8 1.6 26.7 18.2	1st component 2nd component 1st component 2nd component 1st component 2nd component	Kedderis et al., 1991
CDFs						
2,3,7,8-TCDF	Fischer 344 rat (M)	30.6 µg/kg, i.v. (0.1 µmol/kg)	liver adipose skin muscle blood	0.10 1.25 3.75 0.45 11.09 0.02 0.72 0.02 1.14	Pool size (% of total dose) 29.09 1st component 31.39 2nd component 17.85 6.84 1st component 1.22 2nd component 24.85 1st component 6.73 2nd component 1.31 1st component 0.89 2nd component	Birnbaum et al., 1980
2,3,7,8-TCDF	C57BL/6J mice (M)	30.6 µg/kg, i.v. (0.1 µmol/kg)	liver adipose skin muscle	1.9 1.6 0.15 4.0 0.015 1.1	 1st component 2nd component 1st component 2nd component	Decad et al., 1981b
2,3,7,8-TCDF	DBA/2J mice (M)	30.6 µg/kg, i.v. (0.1 µmol/kg)	liver adipose muscle	1.8 7.0 0.02 4.0	 1st component 2nd component	Brewster and Birnbaum, 1988

TABLE 1-5 (cont.)

Chemical	Species (Sex)	Dose	Tissue	Half-Life (days)	Remarks	Reference
1,2,3,7,8-PeCDF	Fischer 344 rat (M)	34 µg/kg, i.v. (0.1 µmol/kg)	liver adipose skin muscle adrenal blood	1.36 25.72 12.91 1.32 14.53 0.03 6.96 0.14 2.36 0.07 12.42	Pool size (% of total dose): 42.59 1st component 1.27 2nd component 10.19 7.14 1st component 1.49 2nd component 34.81 1st component 7.42 2nd component 0.26 1st component 0.02 2nd component 5.33 1st component 1.29 2nd component	Brewster and Birnbaum, 1988
1,2,3,7,8-PeCDF	Sprague-Dawley rat (F)	4.0 µg/kg, p.o.	liver	3.3	69.8% of total dose	Van den Berg et al., 1989
2,3,4,7,8-PeCDF	Fischer 344 rat (M)	34 µg/kg, i.v. (0.1 µmol/kg)	liver adipose skin muscle blood	193 69 0.62 1.23 0.04 0.51 9.84 0.04 1.32 55	Pool size (% of total dose): 67.71 10.53 3.54 1st component 1.37 2nd component 29.40 1st component 2.01 2nd component 0.78 3rd component 3.18 1st component 0.37 2nd component 0.008 3rd component	Brewster and Birnbaum, 1987
2,3,4,7,8-PeCDF	Sprague-Dawley rat (F)	5.6 µg/kg, p.o.	liver	108	78.3% of total dose	Van den Berg et al., 1989
1,2,3,6,7,8-HxCDF	Sprague-Dawley rat (F)	6.0 µg/kg, p.o.	liver	73	63.4% of total dose	Van den Berg et al., 1989
PCBs						
3,3'4,4'-TCB	Sprague-Dawley rat (F)	5 mg/kg/day, p.o., for 21 days,	liver adipose	0.8 2.5	21-Day exposure produced steady state with 300 ng/g in liver and 8 µg/g in adipose tissue. Elimination was assessed over a 22-day post-exposure period.	CLarke et al., 1984

TABLE 1-5 (cont.)

Chemical	Species (Sex)	Dose	Tissue	Half-Life (days)	Remarks	Reference
3,3'4,4'-TCB	ICR mice (M)	8 mg/kg, p.o., every other day for 10 doses	liver adipose serum	2.15 2.60 1.07	Steady state tissue concentrations: 1.5 µg/g 19.2 µg/g 0.04 µg/mL	Clevenger et al., 1989

i.v. = Intravenous; s.c. = subcutaneous; i.p. = intraperitoneal; p.o. = *per os*

for the second component (days 49-91) (see Figure 1-1 and Table 1-5). Results of Abraham et al. (1988) and Lakshmanan et al. (1986) indicate that in the rats, 2,3,7,8-TCDD is more persistent in the adipose tissue than in the liver. This is in contrast to the mouse, where liver and adipose tissue have similar half-lives (Birnbaum, 1986). 2,3,7,8-TCDD is exceptionally persistent in the adipose tissue of the rhesus monkey, with a half-life approximately 10- to 40-fold greater than that observed in the rat and mouse (Bowman et al., 1989). Thus, the relative persistence of 2,3,7,8-TCDD is tissue specific and exhibits marked interspecies variability.

Most of the pharmacokinetic data on the relative persistence of other congeners in Table 1-5 has been reported in rat studies, which limits interspecies comparisons. Results in the rat suggest that the distribution and elimination of 2,3,7,8-TBDD from tissue are similar to that of 2,3,7,8-TCDD. The most persistent congeners are OCDD, 2,3,4,7,8-PeCDF and 1,2,3,6,7,8-HxCDF, which distribute almost entirely to the liver. OCDD and 2,3,4,7,8-PeCDF also exhibit similar elimination kinetics, with a relative half-life in the liver more than 2-fold greater than that in adipose tissue. The least persistent congeners are 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF and 3,3'4,4'-TCB. These congeners exhibit similar elimination kinetics in the rat with half-lives in the adipose tissue greater than those in liver. The relative tissue distribution of these congeners varies, however, with 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF distributing primarily to the liver, while 3,3',4,4'-TCB distributes predominantly to the adipose tissue.

The experimental tissue distribution and elimination data in Table 1-5 were obtained after exposure to a single congener, while real world exposure to 2,3,7,8-TCDD and related compounds occurs as a complex mixture of congeners. Recently, Neubert et al. (1990) examined the persistence of various CDDs and CDFs in hepatic and adipose tissue of male and female marmoset monkeys. Animals received a single subcutaneous exposure to a defined CDD/CDF mixture (total dose of 27.8 μg/kg bw), which contained 0.12 μg 2,3,7,8-TCDD/kg bw. Using the I-TE factors (NATO, 1988; U.S. EPA, 1989), the total administered dose corresponded to 0.464 μg I-TE/kg bw. The concentrations of specific congeners in liver and adipose tissue were measured at 1, 6, 16 or 28 weeks after exposure, and elimination constants and half-lives were estimated assuming first-order kinetics

(Table 1-6). Data in Table 1-6 were determined from pregnant and nonpregnant female and male marmosets (total of 12 animals) since no obvious differences in tissue concentrations were observed among these groups. All 2,3,7,8-substituted CDDs and CDFs were consistently more persistent in the adipose tissue than in the liver of marmoset monkeys. In general, the persistence in adipose tissue was from ~1.3- to 2.0-fold greater than that in liver, with the exception of 1,2,3,4,7,8-/1,2,3,4,7,9-HxCDF, HpCDFs and OCDF, which were even more persistent in adipose tissue. For the latter congeners and OCDD, there was marked variance in half-life values, which may be due to delayed and incomplete absorption of the exceptionally persistent congeners and the relatively short (28 weeks) period of investigation. A significant species difference exists for OCDD and 2,3,4,7,8-PeCDF, which, in contrast to the marmoset monkey, was found to be more persistent in the liver of the rat, with half-lives more than 2-fold greater than that in adipose tissue (Birnbaum and Couture, 1988; Brewster and Birnbaum, 1987) (see Table 1-5). Further comparison of tissue elimination data in the rat (Table 1-5) and monkey (Table 1-6) indicates that 2,3,7,8-TCDD, OCDD, 2,3,7,8-TCDF, 1,2,3,6,7,8-HxCDF and 2,3,4,7,8-PeCDF (adipose tissue only) are more persistent in the marmoset monkey than in the rat. The exception to this relationship is 2,3,4,7,8-PeCDF, which is more persistent in rat liver, compared to the monkey.

The exposure of marmoset monkeys to a complex mixture of CDDs and CDFs included exposure to both 2,3,7,8- and non-2,3,7,8-substituted congeners (Neubert et al., 1990). One week after exposure to this complex mixture, the non-2,3,7,8-substituted CDDs and CDFs were present in liver and adipose tissue in relatively minor quantities when compared with 2,3,7,8-substituted congeners; however, non-2,3,7,8-substituted compounds represented a considerable percent of the exposure mixture. In this study, none of the non-2,3,7,8-substituted TCDDs, PeCDDs, TCDFs or PeCDFs could be detected in the liver. Some of the hexa and hepta congeners were detected in adipose tissue and liver, but after 1 week, the total amount in the liver was >5% of the administered dose only in the case of 1,2,4,6,8,9-HxCDF. Similar results were obtained in rats after exposure to a defined, complex mixture of CDDs and CDFs (Abraham et al., 1989). Additional short-term studies in rats provide evidence that the low tissue concentration of non-2,3,7,8-substituted congeners, measured 1 week after exposure, were the result of rapid

TABLE 1-6

Elimination Constants and Half-Lives of Various 2,3,7,8-Substituted CDDs and CDFs in Hepatic and Adipose Tissue of Marmoset Monkeys[a,b]

Congener	Hepatic Tissue			Adipose Tissue		
	K_e (weeks^{-1})	Half-Life (weeks)	95% Conf. Interval (weeks)	K_e (weeks^{-1})	Half-Life (weeks)	95% Conf. Interval (weeks)
2,3,7,8-TCDD[c]	0.0841±0.0109	8.3	6.6–11.1	0.0658±0.0072	10.5	8.7–13.4
1,2,3,7,8-PeCDD[c]	0.0649±0.0101	10.7	8.2–15.4	0.0490±0.0057	14.2	11.5–18.3
1,2,3,4,7,8-HxCDD	0.0702±0.0059	9.9	8.4–11.8	0.0411±0.0083	16.9	12.1–27.9
1,2,3,6,7,8-HxCDD	0.0558±0.0046	12.4	10.7–14.9	0.0373±0.0073	18.6	13.4–30.2
1,2,3,7,8,9-HxCDD	0.0767±0.0078	9.0	7.5–11.3	0.0525±0.0089	13.2	9.9–19.7
1,2,3,4,6,7,8-HpCDD	0.0518±0.0081	13.4	10.2–19.3	0.0372±0.0060	18.6	14.2–27.2
OCDD	0.0089±0.0084	78	27–∞[d]	0.0122±0.0093	101	20–∞[d]
2,3,7,8-TCDF	0.8012±0.0549	<0.87[e]	-<1.00	0.4986±0.0829	1.39	1.05–2.06
1,2,3,7,8-/1,2,3,4,8-PeCDF	0.7476±0.0294	0.93	0.86–1.00	0.4735±0.0408	1.46	1.25–1.76
2,3,4,7,8-PeCDF	0.0786±0.0048	8.8	7.9–10.0	0.0563±0.0059	12.3	10.2–15.5
1,2,3,4,7,8-/1,2,3,4,7,9-HxCDF	0.0307±0.0039	23	18–30	0.0103±0.0074	68	28–∞[d]
1,2,3,6,7,8-HxCDF	0.0486±0.0037	14.3	12.4–16.7	0.0290±0.0091	24	15–62
1,2,3,7,8,9-HxCDF	0.0848±0.0057	8.2	7.2–9.4	not analyzed[f]	NA	NA
2,3,4,6,7,8-HxCDF	0.0373±0.0057	18.6	14.3–26.5	0.0182±0.0082	38	20–327
1,2,3,4,6,7,8-HpCDF	0.0186±0.0072	37	21–152	-0.0140±0.0137	∞[d]	54–∞[d]

TABLE 1-6 (cont.)

Congener	Hepatic Tissue			Adipose Tissue		
	K_e ($weeks^{-1}$)	Half-Life (weeks)	95% Conf. Interval (weeks)	K_e ($weeks^{-1}$)	Half-Life (weeks)	95% Conf. Interval (weeks)
1,2,3,4,7,8,9-HpCDF	0.0088±0.0127	79	20-∞[d]	0.0011±0.0112	660	30-∞[d]
OCDF	0.0040±0.0096	174	30-∞[d]	-0.0042±0.0148	∞[d]	28-∞[d]

[a]Source: Neubert et al., 1990

[b]Animals were treated subcutaneously with a single dose of a defined CDD\CDF mixture, and the tissues were analyzed at different times following treatment. Half-lives were calculated from tissue concentrations of the 2,3,7,8-substituted congeners in hepatic and adipose tissue. Values are given as elimination rate constant K^e including estimated SD and half-life including 95% confidence intervals.

[c]Calculated from the time period: >6 weeks after injection.

[d]Calculated half-life is apparently infinite. Data for OCDD and OCDF are unreliable due to delayed absorption.

[e]Not detected in hepatic tissue 6 weeks after treatment; limits of detection used for calculation

[f]Due to interference

NA = Not applicable

elimination, since these congeners were detected at higher levels in the liver 13-14 hours after exposure (Abraham et al., 1989). These results in monkeys and rats are compatible with data from analysis of human tissue samples and milk in which the non-2,3,7,8-substituted congeners have also not been shown to be present in significant concentrations, when compared with the 2,3,7,8-substituted congeners (Schecter et al., 1985, 1986; Ryan, 1986; Rappe et al., 1986; Beck et al., 1987, 1988; Thoma et al., 1989).

A potential problem of tissue distribution and elimination studies after exposure to a complex mixture of CDDs and CDFs is the possible interaction of the mixture during the uptake and elimination of specific congeners from tissues. A similar hepatic distribution (~25% of dose) and liver/adipose tissue concentrations ratio (~2) for 2,3,7,8-TCDD were observed in rats 7 days after exposure to 2,3,7,8-TCDD (100 ng/kg bw) when the compound was administered alone or in combination with a large amount of other CDDs/CDFs (total 23,222 ng/kg bw) (Abraham et al., 1988, 1989). This suggests that under these experimental conditions, the tissue distribution of 2,3,7,8-TCDD was not altered when the exposure included a complex mixture of CDDs/CDFs. Van den Berg et al. (1989) studied the hepatic disposition and elimination of CDFs when administered individually (see Table 1-5) and as mixtures. Co-administration of 1,2,3,7,8- and 2,3,4,7,8-PeCDF resulted in 46% of the dose of 1,2,3,7,8-PeCDF distributing to the liver, while 70% was distributed to the liver after administration of the single compound (see Table 1-5). Nevertheless, this combined exposure did not alter the rate of elimination of 1,2,3,7,8-PeCDF from the liver. Co-administration of 2,3,4,7,8-PeCDF and 1,2,3,6,7,8-HxCDF did not alter the hepatic uptake of either congener or the hepatic elimination of 2,3,4,7,8-PeCDF but increased the hepatic half-life of 1,2,3,6,7,8-HxCDF to 156 days from the single compound exposure half-life of 73 days (see Table 1-5). However, these values must be considered rough estimates since the experimental period of 42 days was too short to accurately calculate half-lives. Although there are few investigations of potential interactions of mixtures of CDDs and CDFs on the uptake and elimination of individual congeners, the limited available data suggests that exposure to complex mixtures (see Table 1-6) may alter the tissue disposition of individual

congeners. There is clearly a need for more understanding of possible pharmacokinetic interactions of complex mixtures of these and other compounds.

1.2.4. Dose-Dependent Tissue Distribution. Recent evidence suggests that the tissue distribution of 2,3,7,8-TCDD and related compounds is dose dependent. Abraham et al. (1988) investigated the distribution of 2,3,7,8-TCDD in liver and adipose tissue of rats 7 days after a single subcutaneous exposure to 2,3,7,8-TCDD at doses of 1-3000 ng/kg bw. Greater than 97% of the administered 2,3,7,8-TCDD was absorbed at all doses, with the exception of the 3000 ng/kg group where 84% of the dose was absorbed. Figure 1-2 illustrates the dose-dependent disposition of 2,3,7,8-TCDD in liver and adipose tissue (% dose/g) 7 days after exposure. A sharp increase in 2,3,7,8-TCDD concentration in liver was observed at exposure levels >10 ng/kg bw. Disposition in the liver increased from ~11% of the administered dose at an exposure level of 1-10 ng/kg bw to ~37% of the dose at an exposure level of 300 ng/kg bw. The increase in distribution to the liver was accompanied by a dose-related decrease in the concentration of 2,3,7,8-TCDD in the adipose tissue. As a result, the liver/adipose tissue concentration ratio for 2,3,7,8-TCDD at 7 days after exposure increased with increasing doses, starting at an exposure level of 30 ng/kg bw (Table 1-7). Thus, the tissue-specific disposition of 2,3,7,8-TCDD is regulated by a complex relationship, which includes species, time after a given exposure and dose (see Figures 1-1 and 1-2; Tables 1-5 and 1-6).

Other studies on the tissue disposition of 2,3,7,8-TCDD and related compounds report similar dose-dependent behavior with disproportionally greater concentrations in the liver at high doses compared with low doses. Poiger et al. (1989) observed a dose-related increase in distribution to the liver (% of dose/liver) and/or an increase in the liver/adipose tissue concentration ratio for 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDF and 1,2,3,6,7,8-HxCDF in the rat. Kedderis et al. (1991a) also observed a dose-related increase in hepatic disposition (1.27 versus 10.05 % of dose/liver) and an increase in the liver/adipose tissue concentration ratio (0.16 versus 2.59) for 2,3,7,8-TBDD at 56 days after exposure at doses of 0.001 and 0.1 μmol/kg bw, respectively. In a related study, pretreatment of mice with 2,3,7,8-TCDD (5 or 15 μg/kg) produced a dose-related, enhanced hepatic accumulation of a subsequent oral dose of 2,3,7,8-TCDD

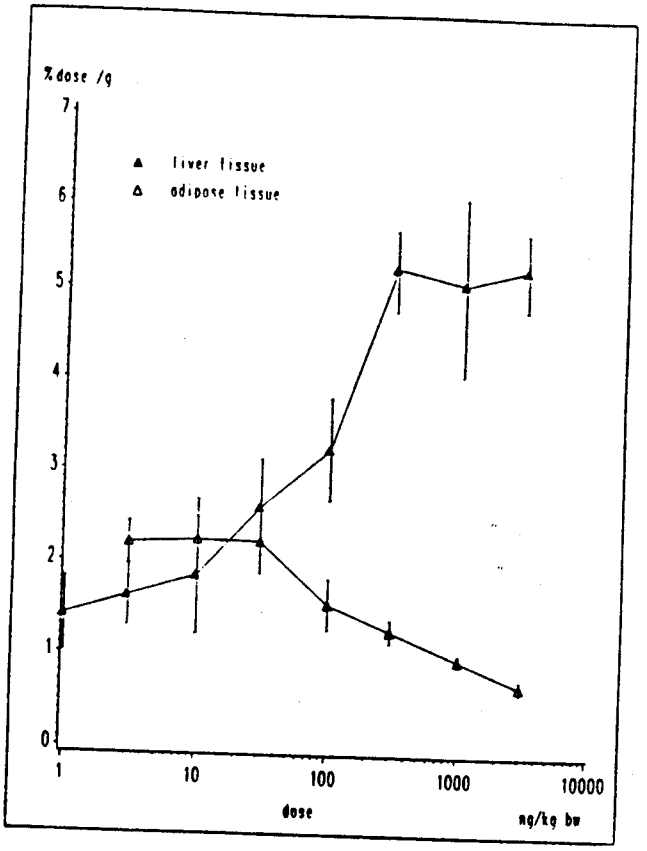

FIGURE 1-2

Dose Dependency of the Percentage of the Administered Dose of ^{14}C-TCDD/g of Tissue Recovered in Liver and Adipose Tissue After Single Subcutaneous Doses (values from animals treated with 3000 ng TCDD/kg bw were corrected for 84% absorption). Concentrations were measured 7 days after the injection.

Source: Abraham et al., 1988

TABLE 1-7

2,3,7,8-TCDD Concentrations in Liver and Adipose Tissue Following Different Doses and Calculated Concentration Ratios (Liver/Adipose Tissue)[a,b]

Dose (ng/kg)	Number	TCDD Concentration Liver (ng/g)	TCDD Concentration Adipose Tissue (ng/g)	Concentration Ratio: Liver/Adipose Tissue
1	6	0.0031±0.0009	ND	NA
3	6	0.0102±0.0020	0.0139±0.0015	0.74±0.15
10	12	0.0406±0.0121	0.0494±0.0084	0.82±0.20
30	6	0.162±0.032	0.139±0.021	1.16±0.07
100	6	0.699±0.130	0.335±0.065	2.10±0.27
300	6	3.38±0.22	0.819±0.075	4.14±0.31
1000	6	10.7±2.2	2.02±0.17	5.27±0.96
3000	5	27.9±2.4	3.66±0.31	7.65±0.64

[a]Source: Abraham et al., 1988

[b]Concentrations were measured 7 days after injection

ND = Not detectable; NA = not applicable

(Curtis et al., 1990). Similarly, a dose-related increase in hepatic uptake of [^{125}I]-2-iodo-3,7,8-trichlorodibenzo-*p*-dioxin was observed after pretreatment of mice with 2,3,7,8-TCDD (Poland et al., 1989; Leung et al., 1990). Shen and Olson (1987) also observed an increase in the uptake of 2,3,7,8-TCDD by isolated hepatocytes from 2,3,7,8-TCDD pretreated mice.

Chronic studies also support dose-dependent alterations in the tissue distribution of these compounds. Kociba et al. (1978a,b) found that female rats maintained on a daily dietary 2,3,7,8-TCDD intake of 100 ng for 2 years had an average 2,3,7,8-TCDD content of 8100 ppt in fat and 24,000 ppt in the liver. Rats given 10 ng/kg/day had an average of 1700 ppt 2,3,7,8-TCDD in the fat and 5100 ppt in the liver. For both of these exposures the liver/ adipose tissue concentration ratio of 2,3,7,8-TCDD was ~3. At the lowest dose level of 1 ng/kg/day, both fat and liver contained an average of 540 ppt 2,3,7,8-TCDD. Kociba et al. (1976) presented evidence that steady state had been reached by <13 weeks of feeding of 2,3,7,8-TCDD.

Other studies do not support the dose-dependent tissue distribution of 2,3,7,8-TCDD and related compounds described above. Rose et al. (1976) reported a lack of a dose-dependent accumulation of ^{14}C-TCDD in male and female rat liver and adipose tissue following 7, 21 and 49 days of exposure at 0.01, 0.1 or 1.0 μg/kg/day, Monday through Friday. The rates of accumulation of TCDD-derived radioactivity were similar in fat, liver and whole body; however, the concentration in the liver was ~5-fold greater than that in fat. Recently, Clark et al. (1991) and Tritscher et al. (1992) also reported a lack of a dose-dependent hepatic disposition of TCDD in female Sprague-Dawley rats exposed biweekly to TCDD for 30 weeks at doses equivalent to 3.5, 10.7, 35.7 and 125 ng/kg/day. A linear relationship between administered dose and the concentration in the liver was observed over the dose range used in this chronic exposure study. Brewster and Birnbaum (1987) also observed similar concentrations (% dose/g) of 2,3,4,7,8-PeCDF in liver, adipose tissue and other tissues at 3 days after oral exposure at doses of 0.1, 0.5 or 1.0 μmol/kg bw. These results conflict with the above studies which support the dose-dependent tissue distribution of these compounds.

While it is not possible at this time to explain these differences, most of the available data support a dose-dependent relationship.

The dose-dependent tissue distribution of 2,3,7,8-TCDD and related compounds is a critical factor that must be considered in estimating the concentration of these compounds in human tissues after chronic low-level exposure. This is particularly important since the general human population is exposed to much smaller daily doses (possibly 0.3 pg 2,3,7,8-TCDD/kg/day) than those used in experimental disposition studies. Due at least partly to the long half-life of 2,3,7,8-TCDD in humans, however, this exposure results in concentrations of 3-18 pg/g in human adipose tissue (Leung et al., 1990). Similar levels of 2,3,7,8-TCDD in adipose tissue (14 pg/g) were observed in rats 7 days after subcutaneous exposure to 3 ng/kg bw (see Table 1-7) (Abraham et al., 1988). Under these experimental conditions, the liver/adipose tissue 2,3,7,8-TCDD concentration was 0.74. Nonetheless, steady state was definitely not reached under these conditions, and, with increasing time after exposure, this ratio may decrease, based on the observation that 2,3,7,8-TCDD was more persistent in adipose tissue than in liver in rats exposed to 300 ng/kg bw (see Figure 1-1 and Table 1-5) (Abraham et al., 1988). Human data on the liver/adipose tissue concentration ratio of 2,3,7,8-TCDD and related compounds are limited but suggest that the ratio may vary by at least an order of magnitude between individuals. Leung et al. (1990) observed a geometric mean adipose tissue 2,3,7,8-TCDD concentration of 7.78 ppt in 26 individuals and a concentration in liver at about one-tenth of that in adipose tissue on a whole weight basis. When measured on a total lipid basis, the concentrations of 2,3,7,8-TCDD in both tissues were approximately the same. More variability between individuals was observed in the CDD and CDF concentrations in liver and adipose tissue from 25 subjects from the Munich area (Thoma et al., 1989). For example, the liver/adipose tissue concentration ratio for 2,3,7,8-TCDD was >1.0 for 5 of the 25 individuals (Thoma et al., 1989). While the majority of individuals had liver/adipose tissue concentration ratios <1.0 for CDDs and CDFs, ratios >1.0 were observed for HpCDD (5 of 25), OCDD (2 of 25), PeCDF (2 of 25), HxCDF (1 of 25), HpCDF (7 of 25) and OCDF (3 of 25). Considerable variability in CDD and CDF concentrations in liver and adipose tissues was also observed between individual marmoset monkeys (Neubert et al.,

1990), suggesting that individual variability may also contribute to the difficulty in assigning a constant liver/adipose tissue ratio for CDDs and CDFs in humans and nonhuman primates.

1.2.5. Potential Mechanisms for the Dose-Dependent Tissue Distribution. The observation that exposure to higher doses of 2,3,7,8-TCDD and related compounds results in a disproportionally greater hepatic concentration of these compounds may be explained by a hepatic binding species that is induced by 2,3,7,8-TCDD and other agonists for the Ah receptor. The studies of Voorman and Aust (1987, 1989) and Poland et al. (1989a,b) provide evidence that this binding species is cytochrome P-4501A2.

Poland et al. (1989) reported that TCDD and other Ah agonists (2,3,7,8-TCDF, ß-naphthoflavone, 3,3',4,4',5,5'-hexabromobiphenyl) act through the Ah receptor to increase a liver binding species that increases the hepatic uptake of [^{125}I]-2-iodo-3,7,8-trichlorodibenzo-*p*-dioxin (a radiolabeled isosteric analogue of TCDD) *in vivo* and binding of this radioligand to liver homogenate *in vitro*. Twenty-four hours after the administration of a non-AHH-inducing dose ($1x10^{-10}$ mol/kg) of [^{125}I]-2-iodo-3,7,8-trichlorodibenzo-*p*-dioxin to C576BL/6J mice, the hepatic concentration of radioactivity was 1-2% of the administered dose, whereas in mice pretreated 48 hours earlier with an AHH inducing dose of TCDD ($1x10^{-7}$ mol/kg), the hepatic accumulation of radiolabel was 25-30% of that administered. A similar, though less dramatic effect, was observed *in vitro*, with liver homogenate from TCDD-treated mice binding about four times more [^{125}I]-2-iodo-3,7,8-trichlorodibenzo-*p*-dioxin than homogenate from control mice. The administration of TCDD to C57BL/6J mice produced a dose-related stimulation of *in vivo* hepatic uptake of [^{125}I]-2-iodo-3,7,8-trichlorodibenzo-p-dioxin, increased binding of radioligand to liver homogenate and induction of hepatic activity, with an ED_{50} ranging from 1.5 to $4.0x10^{-9}$ mol/kg. In congenic C57BL/6J (Ah^d/Ah^d) mice, which express the lower affinity Ah receptor, the ED_{50} values for all three responses were shifted to doses that were about 10-fold higher. The observed effects on hepatic disposition were tissue specific, with no remarkable dispositional changes being observed in kidney, lung, spleen, small intestine or muscle.

This is significant in that TCDD and other agonists for the Ah receptor induce cytochrome P-4501A1 in liver and other tissues, whereas cytochrome P-4501A2 is apparently inducible only in liver and nasal olfactory mucosa (Tuteja et al., 1985; Gillner et al. 1987). Furthermore, the changes in hepatic disposition were not species specific; similar responses were observed in guinea pigs, rats, mice and hamsters (Poland et al., 1989).

The following evidence reported by Poland et al. (1989) supports the hypothesis that the TCDD-inducible hepatic binding protein is cytochrome P-4501A2: the TCDD-induced hepatic binding species was found predominantly in the microsomal fraction and was inactivated by heating at 60°C, trypsin and mercurials; the TCDD-induced hepatic binding species was specific for the liver, with a large pool size (B_{max} of 22±5 nmol/g liver); and the major microsomal binding species covalently labeled with the photo-affinity ligand [^{125}I]-2-iodo-3-azido-7,8-dibromodibenzo-*p*-dioxin migrates with that immunochemically stained with polyclonal antiserum that binds to cytochrome P-4501A2.

One observation of Poland et al. (1989) does not support the hypothesis that the TCDD-inducible hepatic protein is cytochrome P-4501A2. These investigators found that dietary administration of isosafrole did not stimulate hepatic uptake of [^{125}I]-2-iodo-3,7,8-trichlorodibenzo-*p*-dioxin or the *in vitro* binding of this ligand to liver homogenate. Isosafrole is not an agonist for the Ah receptor, but it selectively induces cytochrome P-4501A2 (Ryan et al., 1980). Poland et al. (1989) suggest that this may be attributable to the high affinity binding of an isosafrole metabolite to the protein, which might inhibit the binding of [^{125}I]-iodo-3,7,8-trichlorodibenzo-*p*-dioxin to cytochrome P-4501A2 at or near the active site of the enzyme. This does not explain why TCDD, which also has high affinity for cytochrome P-4501A2, cannot displace some of the isosafrole metabolite from the protein, which should produce enhanced hepatic disposition of TCDD.

Voorman and Aust (1987, 1989) support further the hypothesis that cytochrome P-4501A2 is the TCDD-inducible hepatic binding species. These investigators found that 3,3'4,4'5,5'-HxBB, an agonist for the Ah receptor, was associated only with cytochrome P-4501A2 through the immunoprecipitation of cytochromes P-4501A1

and 1A2, which were induced in 3,3'4,4'5,5'-HxBB treated rats. In addition, they found that 3,3'4,4'5,5'-HxBB inhibited estradiol 2-hydroxylase activity of purified cytochrome P-4501A2. A similar association of PAHs with immunoprecipitated cytochrome P-4501A2 was observed for other agonists for the Ah receptor, including 2,3,7,8-TCDD, 3,3',4,4'-TCB, 3,3',4,4',5-PeCB and 3,3',4,4',5,5'-HxCB. The association of 2,3,7,8-TCDD with cytochrome P-4501A2 occurred within 2 minutes, with maximum inhibition of estradiol 2-hydroxylase occurring at a concentration comparable to the concentration of the enzyme (50 nm). Cytochrome P-4501A2 was inhibited (complexed) by 2,3,7,8-TCDD with nearly 1:1 stoichiometry and the K_i for 2,3,7,8-TCDD was calculated to be 8 nM. Therefore, 2,3,7,8-TCDD can be considered a higher binding inhibitor of cytochrome P-4501A2.

The TCDD-induced binding species was found to have an apparent equilibrium dissociation constant, K_D, [^{125}I]-2-iodo-3,7,8-trichlorodibenzo-*p*-dioxin of 56±16 nM and a pool size, B_{max}, of 22±5 nmol/g of liver in C57BL/6J mice (Poland et al., 1989). The induced microsomal binding species has an affinity about 10^4 times less than the Ah receptor but a pool size that is ~$2x10^3$ greater. Thus, agonists for the Ah receptor may significantly affect their disposition through a dose-related enhancement of hepatic uptake which should correlate with induction of cytochrome P-4501A2.

The disposition and pharmacokinetics of 2,2',4,4',5,5'-HxCB and -HxBB have been investigated in several species (Tuey and Matthews, 1980; Lutz et al., 1984). These lipophilic compounds are similar to 2,3,7,8-TCDD in that they are slowly metabolized and that metabolism is required for urinary and biliary elimination. 2,2',4,4',5,5'-HxCB and -HxBB distribute primarily to adipose tissue with partition coefficients (tissue/blood ratio) ranging from 300-500 in the mouse, rat, monkey, dog and human. The liver is not a major site for the disposition of 2,2',4,4',5,5'-HxCB and -HxBB, in contrast to 2,3,7,8-TCDD and related compounds. Partition coefficients in the liver range from 10-30 in these species. 2,2',4,4',5,5'-HxCB and -HxBB do not induce hepatic cytochrome P-4501A1 or 1A2 and do not exhibit dioxin-like activity. The lack of induction of hepatic cytochrome P-4501A2 may explain the lack of a dose-dependent hepatic disposition of these compounds.

Kedderis et al. (1991b) assessed the dose-response relationship for the induction of hepatic cytochrome P-4501A1 and P-4501A2 in male Fischer 344 rats exposed to 2,3,7,8-TBDD at doses as low as 0.1 nmol/kg. They reported that induction of P-4501A2 by 2,3,7,8-TBDD appeared to be a more sensitive response than P-4501A1 induction over the dose-range studied. In addition, comparison of hepatic P-4501A2 levels and liver:adipose tissue concentration ratios suggested that induction of P-4501A2 alone would not directly account for the preferential hepatic accumulation of 2,3,7,8-TBDD, and additional factors must be involved. One explanation may be that at low 2,3,7,8-TBDD concentrations, endogenous substrates bind to CYP1A2, not allowing 2,3,7,8-TBDD to be sequestered by the protein (Birnbaum, 1992). At higher 2,3,7,8-TBDD concentrations, new protein is formed and 2,3,7,8-TBDD can compete for binding to CYP1A2, resulting in the increased hepatic deposition observed at higher exposures of 2,3,7,8-TBDD (Kedderis et al., 1991a).

Other factors may also regulate the intracellular distribution of 2,3,7,8-TCDD and related compounds. The possible role of hepatic lipoproteins as intracellular carriers in the transport of 2,3,7,8-TCDD has been assessed by *in vitro* and *in vivo* studies (Souès et al., 1989a,b). 2,3,7,8-TCDD and 2,3,7,8-TCDF were bound to lipoproteins in mouse and rat liver, which subsequently underwent rapid and pronounced degradative processing, possibly catalyzed by lipoprotein lipase, to heavier entities. The *in vitro* incubation of 2,3,7,8-TCDD-lipoprotein complex with separated Ah receptor demonstrated that a passive transfer occurred. The authors suggest a carrier-role for lipoproteins in the intracellular transport of 2,3,7,8-TCDD and related compounds.

1.3. METABOLISM AND EXCRETION

Although early *in vivo* and *in vitro* investigations were unable to detect the metabolism of 2,3,78-TCDD (Vinopal and Casida, 1973; Van Miller et al., 1976), there is evidence that a wide range of mammalian and aquatic species are capable of biotransforming 2,3,7,8-TCDD to polar metabolites (Ramsey et al., 1979, 1982; Poiger and Schlatter, 1979; Olson et al., 1980; Olson, 1986; Gasiewicz et al., 1983; Poiger et al., 1982; Sijm et al., 1990; Kleeman et al., 1986a,b, 1988). Although metabolites of 2,3,7,8-TCDD have not been directly identified in humans,

recent data regarding feces samples from humans in a self-dosing experiment suggests that humans can metabolize 2,3,7,8-TCDD (Wendling et al., 1990).

Table 1-8 summarizes data on the metabolism and excretion of 2,3,7,8-TCDD and related compounds after exposure to a single radiolabeled congener. Investigations of 2,3,7,8-TCDD in rats, mice, guinea pigs and hamsters found that >90% of the radiolabeled material excreted in urine and bile represented polar metabolites. Similar results were also observed for other congeners (see Table 1-8), with the exception of OCDD; however, although studies were often limited to the rat. OCDD is apparently not metabolized by the rat or metabolized to a very minimal extent (Birnbaum and Couture, 1988). For all of the congeners in Table 1-8, essentially all of the CDD, BDD, CDF or PCB-derived radioactivity in liver, adipose tissue and other tissues represented parent compound, suggesting that the metabolites of these compounds were readily excreted. Thus, with the exception of OCDD, the metabolism of 2,3,7,8-TCDD and related compounds is required for urinary and biliary elimination and therefore plays a major role in regulating the rate of excretion of these compounds. In addition, direct intestinal excretion of parent compound is another route for excretion of 2,3,7,8-TCDD and related compoundst that is not regulated by metabolism.

1.3.1. Structure of Metabolites. Several metabolites of 2,3,7,8-TCDD have recently been identified. Sawahata et al. (1982) investigated the *in vitro* metabolism of 2,3,7,8-TCDD in isolated rat hepatocytes. The major product was deconjugated with ß-glucuronidase, derivatized with diazomethane and separated into two compounds by HPLC. These metabolites were subsequently identified as 1-hydroxy-2,3,7,8-TCDD and 8-hydroxy-2,3,7-trichlorodibenzo-*p*-dioxin. Poiger et al. (1982b) identified six metabolites in the bile of dogs that were given a lethal dose of [^{3}H]-2,3,7,8-TCDD. The major metabolite was 1,3,7,8-tetrachloro-2-hydroxydibenzo-*p*-dioxin; however, 3,7,8-trichloro-3-hydroxydibenzo-*p*-dioxin and 1,2-dichloro-4,5-hydroxybenzene were identified as minor metabolites. The structures of the three remaining metabolites were not determined; however, two appeared to be trichlorohydroxydibenzo-*p*-dioxins and the third was apparently a chlorinated 2-hydroxydiphenyl ether. Poiger and Buser (1984) reported differences in the relative amounts of various 2,3,7,8-TCDD metabolites in dog and rat

TABLE 1-8

Metabolism and Excretion of 2,3,7,8-TCDD and Related Compounds[a]

Chemical	Species	Dose	Chemical Nature of Excretion Products (% Metabolites)			Ratio of % of Dose Excreted (Feces/Urine)	Half-Life[b] (days)	Comment	Reference
			Urine	Bile	Feces				
CDDs									
2,3,7,8-TCDD	Sprague-Dawley rat (M)	50 µg/kg, p.o.	NA	NA	NA	4.0	17.4±5.6[c]	NC	Piper et al., 1973
2,3,7,8-TCDD	Sprague-Dawley rat (M)	7 or 72 ppb in diet for 42 days	NA	NA	NA	NA	12	NC	Fries and Morrow, 1975
2,3,7,8-TCDD	Sprague-Dawley rat (F)	7 or 72 ppb in diet for 42 days	NA	NA	NA	NA	15	NC	Fries and Morrow, 1975
2,3,7,8,-TCDD	Sprague-Dawley rat (M, F)	1.0 µg/kg, p.o	NA	NA	NA	9.9	31±6[d]	NC	Rose et al., 1976
2,3,7,8-TCDD	Sprague-Dawley rat (M, F)	0.1 and 1.0 µg/kg/day, 5 days/week for 7 weeks	NA	NA	NA	8.5	23.7	NC	Rose et al., 1976
2,3,7,8-TCDD	Han/Wistar rat (M)	5 µg/kg, i.p.	>90	NA	~70-90	14.1	21.9	NC	Pohjanvirta et al., 1990
2,3,7,8-TCDD	Long-Evans rat (M)	5 µg/kg, i.p.	>90	NA	~20-90	12.0	20.8	NC	Pohjanvirta et al., 1990
2,3,7,8-TCDD	Sprague-Dawley (M)	500 µg/kg, i.p.	100	100	NA	NA	NA	NC	Neal et al., 1982
2,3,7,8-TCDD	C57BL/6J mice (M)	10 µg/kg, i.p.	100	100	85	2.7	11.0±1.2[d]	NC	Gasiewicz et al., 1983
2,3,7,8-TCDD	DBA/2J mice (M)	10 µg/kg, i.p.	100	100	82	1.2	24.4±1.0[d]	NC	Gasiewicz et al., 1983
2,3,7,8-TCDD	B6D2F1J mice (M)	10 µg/kg, i.p.	100	100	86	2.5	12.6±0.8[d]	NC	Gasiewicz et al., 1983

TABLE 1-8 (cont.)

Chemical	Species	Dose	Chemical Nature of Excretion Products (% Metabolites)			Ratio of % of Dose Excreted (Feces/Urine)	Half-Life[b] (days)	Comment	Reference
			Urine	Bile	Feces				
2,3,7,8-TCDD	C57BL/6J mice Ah^b/Ah^d (M)	500 ng/kg, i.p.	NA	NA	NA	3.1	9.42	NC	Birnbaum, 1986
2,3,7,8-TCDD	C57BL/6J mice Ah^d/Ah^d (M)	500 ng/kg, i.p.	NA	NA	NA	2.1	9.74	NC	Birnbaum, 1986
2,3,7,8-TCDD	DBA/2J Ah^b/Ah^d (F)	500 ng/kg, i.p.	NA	NA	NA	5.3	10.40	NC	Birnbaum, 1986
2,3,7,8-TCDD	DBA/2J Ah^d/Ah^d (F)	500 ng/kg, i.p.	NA	NA	NA	6.8	11.11	NC	Birnbaum, 1986
2-Iodo-3,7,8-TCDD	C57BL/6J mice (F)	[^{125}I] 0.1 nmol/kg, i.p.	NA	NA	NA	NA	14.2	whole body counting was used to estimate body burden over 30-day period	Leung et al., 1990
2-Iodo-3,7,8-TCDD	C57BL/6J mice (F)	[^{125}I] 0.1 nmol/kg, i.p., 3 days following pretreatment with 2,3,7,8-TCDD (0.1 µmol/kg, i.p.)	NA	NA	NA	NA	8.0	whole body counting was used to estimate body burden over 30-day period	Leung et al., 1990
2,3,7,8-TCDD	Hartley guinea pig (M)	0.5 µg/kg, i.p.	NA	NA	NA	15.7	30.2±5.8[d]	NC	Gasiewicz and Neal, 1979
2,3,7,8-TCDD	Hartley guinea pig (M)	0.56 µg/kg, i.p.	100	100	19	11.2	93.7±15.5[d]	NC	Olson, 1986
2,3,7,8-TCDD	Golden Syrian hamster (M)	[^{3}H] 650 µg/kg, i.p.	NA	NA	NA	1.4	11.95±1.95[d]	NC	Olson et al., 1980; Neal et al., 1982
2,3,7,8-TCDD	Golden Syrian hamster (M)	[^{14}C] 650 µg/kg, i.p.	100	100	55-75	NA	10.82±2.35	NC	Olson et al., 1980; Neal et al., 1982

TABLE 1-8 (cont.)

Chemical	Species	Dose	Chemical Nature of Excretion Products (% Metabolites)			Ratio of % of Dose Excreted (Feces/Urine)	Half-Life[b] (days)	Comment	Reference
			Urine	Bile	Feces				
2,3,7,8-TCDD	Golden Syrian hamster (M)	[^{3}H] 650 µg/kg, p.o.	NA	NA	NA	NA	14.96±2.53	NC	Olson et al., 1980; Neal et al., 1982
2,3,7,8-TCDD	human (M)	1.14 ng/kg, p.o.	NA	NA	~50	>3.1	2120[e]	NC	Poiger and Schlatter, 1986; Wendling et al., 1990
2,3,7,8-TCDD	rainbow trout	494 ppt in diet for 13 weeks	NA	~75	NA	NA	105	elimination followed for 13 weeks following exposure	Kleeman et al., 1986
2,3,7,8-TCDD	yellow perch	494 ppt in diet for 13 weeks	NA	~90	NA	NA	126	elimination followed for 13 weeks following exposure	Kleeman et al., 1986
1,2,3,7,8-PeCDD	Sprague-Dawley rat (M, F)	8.42-10.06 µg/kg, p.o.	NA	100	NA	12	29.5±2.7	NC	Wacker et al., 1986
OCDD	Fischer 344 rat (M)	50 µg/kg, iv	<33	0	0	>65	~70	whole body $t_{1/2}$ estimated from body burden in liver, skin and adipose tissue over 56-day period	Birnbaum and Couture, 1988
OCDD	Fischer 344 rat (M)	50 µg/kg/day, p.o., for 10 days	NA	NA	NA	NA	~173	whole body $t_{1/2}$ estimated from body burden in liver, skin and adipose tissue over 112-day period	Birnbaum and Couture, 1988
BDDs									
2,3,7,8-TBDD	Fischer 344 rat (M)	0.001 µmol/kg, iv	NA	100	80-90	11.1	0.7 2.9 17.8	Pool size (% of dose): 11.63 1st component 2.78 2nd component 1.45 3rd component	Kedderis et al., 1991

TABLE 1-8 (cont.)

Chemical	Species	Dose	Chemical Nature of Excretion Products (% Metabolites)			Ratio of % of Dose Excreted (Feces/Urine)	Half-Life[b] (days)	Comment	Reference
			Urine	Bile	Feces				
2,3,7,8-TBDD	Fischer 344 rat (M)	0.1 µmol/kg, iv	NA	100	80-90	9.2	0.4 17.8	Pool size (% of dose): 22.47 1st component 2.35 2nd component	Kedderis et al., 1991
CDFs									
2,3,7,8-TCDF	Fischer 344 rat (M)	0.1 µmol/kg, iv	100	>96	99	31.4	1.8 0.3	fecal excretion urinary excretion	Birnbaum et al., 1980
2,3,7,8-TCDF	C57BL/6J mice (M)	0.1 µmol/kg, iv	100	NA	80	6.5	2.8 1.8 2.0	urine feces urine and feces	Decad et al., 1981b
2,3,7,8-TCDF	DBA/2J mice (M)	0.1 µmol/kg, iv	100	NA	80	2.8	4.9 5.4 4.0	urine feces urine and feces	Decad et al., 1981b
2,3,7,8-TCDF	Hartley guinea pig (M)	0.02 µmol/kg, iv	>90	NA	<10	1.0	20	animal exhibited body weight loss	Decad et al., 1981a
2,3,7,8-TCDF	Hartley guinea pig (M)	4 µg/kg, p.o.	NA	NA	NA	NA	40	no observable toxicity	Ioannou et al., 1983
2,3,7,8-TCDF	rhesus monkey (M)	0.1 µmol/kg, iv	100	>92	>92	5.4	6.24 10.30 ~8	urine feces urine and feces	Birnbaum et al., 1981
1,2,3,7,8-PeCDF	Fischer 344 rat (M)	0.1 µmol/kg, iv	~90	100	NA	12.8	0.92 3.32 1.26 17.32 1.12 6.30	Pool size (% of dose): feces: 57.79 1st component 6.92 2nd component urine: 2.68 1st component 0.16 2nd component feces and urine: 59.97 1st component 2.51 2nd component	Brewster and Birnbaum, 1988
2,3,4,7,8-PeCDF	Fischer 344 rat (M)	0.1 µmol/kg, iv	NA	>90	>90	>100	1.27 63.82	Pool size (% of dose): feces 1.22 1st component 0.57 2nd component	Brewster and Birnbaum, 1987

TABLE 1-8 (cont.)

Chemical	Species	Dose	Chemical Nature of Excretion Products (% Metabolites)			Ratio of % of Dose Excreted (Feces/Urine)	Half-Life[b] (days)	Comment	Reference
			Urine	Bile	Feces				
2,3,4,7,8-PeCDF	rhesus monkey (M)	0.1 µmol/kg, iv	NA	NA	63–70	~34	38–49	$t_{1/2}$ represents minimum value; all animals lost body weight and exhibited other signs of toxicity	Brewster et al., 1988
CBs									
3,3'4,4'-TCB	CD rat (M, F)	0.6 mg/kg, iv	>90	NA	>90	42	~1.3–1.5	NC	Abdel-Hamid et al., 1981
3,3'4,4'-TCB	rhesus monkey (F)	0.6 mg/kg, iv	97	NA	97	7.2	~8–10	NC	Abdel-Hamid et al., 1981

[a]All studies measure the excretion of radiolabeled parent compound and metabolites following exposure to a single congener labeled with ^{3}H, ^{14}C or ^{125}I.

[b]Half-life for excretion estimates assume first-order elimination kinetics

[c](mean±SE)

[d](mean±SD)

[e]n=1

i.p. = Intraperitoneal; i.v. = intravenous; NA = not available; NC = no comment; p.o. = *per os*

bile. Trichlorodihydroxydibenzo-*p*-dioxin and tetrachlorodihydroxydiphenyl ether appear to be major metabolites in rat bile. Furthermore, conjugates, presumably glucuronides, were formed in the rat but not in the dog. The investigators also observed a generally higher metabolism rate of 2,3,7,8-TCDD in the dog. This is in good agreement with the unique ability of the dog to readily metabolize persistent PCBs such as 2,4,5,2'4'5'-HxCB (Sipes et al., 1982).

Biliary metabolites of 2,3,7,8-TCDF have been investigated by Poiger et al. (1984); however, unequivocal structure assignment of the metabolites could not be made using GC/MS. With the use of synthetic standards and GC/MS, Burka et al. (1990) identified 4-hydroxy-2,3,7,8-TCDF and 3-hydroxy-2,7,8-TCDF as major biliary metabolites of 2,3,7,8-TCDF in rats. Small amounts of 3-hydroxy-2,4,7,8-TCDF and 2,2'-dihydroxy-4,4',5,5'-TCB were also detected. This suggests that the preferred site of metabolism of 2,3,7,8-TCDF is near the furan oxygen with oxygenation at C4 predominating over C3. The authors speculate that epoxidation of the C4-C4a bond or the C3-C4 bond could lead to formation of the above metabolites. The results of Burka et al. (1990) and Sawahata et al. (1982) suggest that oxygenation of the unsubstituted carbon nearest the bridging oxygen in both 2,3,7,8-TCDF and 2,3,7,8-TCDD is the major route of metabolism of these compounds in the rat. Furthermore, data on the rate of elimination of these compounds summarized in Tables 1-5 and 1-8 indicate that this reaction occurs at a faster rate for the furan, since the rate of urinary and biliary elimination and resulting persistence of these compounds depends on metabolism.

Data summarized in Tables 1-5 and 1-8 indicate that 1,2,3,7,8-PeCDF is metabolized and eliminated at a greater rate than 2,3,4,7,8-PeCDF. The preference for oxygenation at C4 in 2,3,7,8-TCDF offers an explanation for the observation that 2,3,4,7,8-PeCDF is metabolized at a much slower rate than 1,2,3,7,8-PeCDF, because one of the preferred sites for metabolism is blocked in the 2,3,4,7,8-substituted compound. The rate of metabolism of these compounds and their resulting relative persistence in rodents correlate with analysis of human tissues from the Yusho cohort where the relative concentrations were 2,3,4,7,8-PeCDF > 1,2,3,7,8-PeCDF > 2,3,7,8-TCDF (Masuda et al., 1985).

Pluess et al. (1987) investigated the structure of 1,2,3,7,8-PeCDF metabolites in rat bile. A dihydroxy-tetra-CDF was identified as the major metabolite.

The authors propose that this compound could be formed either via further oxidation of the hydroxy-tetra-CDF or possibly via hydrolytic dechlorination of a hydroxy-penta-CDF. Minor metabolites include a dihydroxy-tri-CDF, hydroxy-tetra-CDF and hydroxy-penta-CPF.

Pluess et al. (1987) also investigated the metabolites of 2,3,4,7,8-PeCDF in rat bile. A total of 10 metabolites were detected with a dihydroxy-penta-CB and a hydroxy-penta-CDF representing the major metabolites. The biphenyl metabolite indicates that cleavage of the ether bridge of the furan is an important pathway for metabolism of this congener. Other less abundant metabolites of 2,3,4,7,8-PeCDF include a hydroxy-tetra-CDF, dihydroxy-tri-CDF, dihydroxy-tetra-CDF and a thio-tetra-CDF. Sulfur-containing metabolites were also identified as minor metabolites of 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF in rats (Kuroki et al., 1990). These sulfur-containing metabolites probably arise from CDF-glutathione conjugates.

In another study, a dihydroxy-PeCDF was identified as the only detectable biliary metabolite of 1,2,3,6,7,8-HxCDF, while no metabolites of 1,2,3,4,6,7,8-HpCDF were detected in the bile of rats that were treated with this congener (Poiger et al., 1989).

Several *in vivo* and *in vitro* studies have investigated the metabolism of 3,3',4,4'-TCB. Rat feces were found to contain 5-hydroxy-3,3',4,4'-TCB and 4-hydroxy-3,3'4',5-TCB as major metabolites (Yoshimura et al., 1987) and 2,5-dihydroxy-3,3',4,4'-TCB, 4,4'-dihydroxy-3,3'5,5'-TCB, 5,6-dihydroxy-3,3',4,4'-TCB, 4-hydroxy-3,3',4-TCB and 4-hydroxy-4',5'-epoxy-3,3'4',5-TCB as minor metabolites (Koga et al., 1989).

Mouse feces were found to contain 5-hydroxy- and 6-hydroxy-3,3',4,4'-TCB and 4-hydroxy-3,3',4'5-TCB while urine contained 2-hydroxy-3,3',4,4'-TCB in addition to these metabolites (Wehler et al., 1989). 3,3'4,4'-TCB was the major compound present in mouse liver, while a minor portion was due to 4-hydroxy-3,3'4,4'-TCB (Wehler et al., 1989). Darnerud et al. (1986) found 2-hydroxy-3,3'4,4'-TCB and a methylsulphonyl-TCB as major metabolites in the mouse fetus. Sulphur-containing metabolites of noncoplanar PCBs have also been reported to accumulate in the bronchial mucosa and uterine luminal fluid of mice (Bergman et al., 1979; Brandt et al., 1982) and in human lung, liver and adipose tissue (Haraguchi et

al., 1986, 1989). PCB methyl sulphones are stable lipophilic metabolites formed by the mercapturic acid pathway. The toxicological significance of these metabolites remains generally unknown.

1.3.2. Toxicity of Metabolites. The above discussion indicates that the metabolism of 2,3,7,8-TCDD and related compounds is required for urinary and biliary elimination and thus plays a major role in regulating the rate of excretion of these compounds. At present, metabolism is also generally considered a detoxification process.

Data on the metabolism of 2,3,7,8-TCDD suggests that reactive epoxide intermediates may be formed. Poland and Glover (1979) have investigated the *in vivo* binding of [1,2-^{3}H]-2,3,7,8-TCDD derived radioactivity to rat hepatic macromolecules, and found maximum levels equivalent to 60 pmol of 2,3,7,8-TCDD/mol of nucleotide in RNA, and 6 pmol of 2,3,7,8-TCDD/mol of nucleotide in DNA. This corresponds to one 2,3,7,8-TCDD-DNA adduct/35 cells. These investigators suggest that it is unlikely that 2,3,7,8-TCDD-induced oncogenesis is through a mechanism of covalent binding to DNA and somatic mutation. Further studies of 2,3,7,8-TCDD and related compounds are needed to confirm these results and assess the relationship between covalent binding and the short and long-term toxicity of these compounds.

Weber et al. (1982) investigated the toxicity of 2,3,7,8-TCDD metabolites by administering extracts of bile from 2,3,7,8-TCDD-treated dogs to male guinea pigs in single oral doses equivalent to 0.6, 6.0 and 60 μg/kg of parent compound. Other groups of guinea pigs were given bile extract form untreated dogs or 2,3,7,8-TCDD itself. A comparison of the mortality data at 5 weeks after dosing indicated that the acute toxicity of 2,3,7,8-TCDD to guinea pigs was at least 100 times higher than was the acute toxicity of its metabolites.

Mason and Safe (1986) synthesized 2-hydroxy-3,7,8-TCDD and 2-hydroxy-1,3,7,8-TCDD, which are metabolites of 2,3,7,8-TCDD, and assessed the toxicity of these compounds in male Wistar rats. The compounds produced no significant effect on body weight gain, thymus, liver or spleen weights after exposure to a dose of ≤5000 μg/kg bw. 2-Hydroxy-3,7,8-TCDD induced hepatic microsomal AHH, EROD and 4-chlorobiphenylhydroxylase activity at an exposure of 1000 and

5000 μg/kg bw, while 2-hydroxy-1,3,7,8-TCDD was inactive as an inducer. Thus, while 2-hydroxy-3,7,8-TCDD has dioxin-like activity as an inducer of the hepatic monooxygenase system, the potency of the metabolite is more than three orders of magnitude less than that of 2,3,7,8-TCDD. Furthermore, results are consistent with the expected rapid conjugation and excretion of these 2,3,7,8-TCDD metabolites (Weber et al., 1982).

Metabolism of coplanar PCBs and PBBs also appears to be a detoxification process. 5-Hydroxy-3,3',4,4'-TCB and 4-hydroxy-3,3',4',5-TCB did not produce liver hypertrophy, induction of hepatic AHH or DT-diaphorase activities or thymus atrophy (Yoshimura et al., 1987). Thus, monohydroxy metabolites of 3,3',4,4'-TCB are much less toxic than the parent compound. Further evidence for metabolism as a detoxification process comes from comparison of the metabolism and toxicity of two coplanar PBBs. Millis et al. (1985) found that 3,3',4,4',5,5'-HxBB exhibited greater toxic potency in rats than 3,3',4,4'-TBB, even though 3,3',4,4'-TBB had about a 10-fold greater affinity for the Ah receptor. Although receptor binding affinities imply that 3,3'4,4'-TBB should be more toxic than 3,3',4,4'5,5'-HxBB, it was less toxic than the HxBB because 3,3',4,4'-TBB was metabolized at a much greater rate than 3,3',4,4',5,5'-HxBB. In addition to supporting metabolism as a detoxification process, the results of Millis et al. (1985) also suggest that receptor binding and *in vitro* AHH induction do not accurately reflect toxicity for PAHs, which are more readily metabolized, presumably because continued occupation of the receptor is required for toxicity.

Structure-activity studies of 2,3,7,8-TCDD and related compounds support the widely accepted principle that this parent compound is the active species. The relative lack of activity of readily excreted monohydroxylated metabolites of 2,3,7,8-TCDD and 3,3'4,4'-TCB suggests that metabolism is a detoxification process necessary for the biliary and urinary excretion of these compounds. This concept has also been generally applied to 2,3,7,8-TCDD related compounds, although data are lacking on the structure and toxicity of metabolites of other CDDs, BDDs, CDFs, BDFs, PCBs and PBBs.

It is possible that low levels of unextractable and/or unidentified metabolites may contribute to one or more of the toxic responses of 2,3,7,8-TCDD

and related compounds. Further studies on the nature of the biotransformation products of these compounds will help to address this uncertainty.

1.3.3. Autoinduction of Metabolism. Accurate rate constants for metabolism are important in developing pharmacokinetic models that describe the disposition of 2,3,7,8-TCDD and related compounds. Metabolism plays a major role in regulating the excretion and relative persistence of these compounds, since metabolism is required for urinary and biliary excretion. Although the relative rate of metabolism of 2,3,7,8-TCDD and related compounds can be estimated from tissue and excretion half-life data (see Tables 1-5 and 1-8), other factors such as relative body composition, hepatic and extrahepatic binding proteins and direct intestinal elimination of the parent compound can also regulate the excretion of 2,3,7,8-TCDD and related compounds. Therefore, *in vivo* disposition data (see Tables 1-5 and 1-8) provide only a limited approximation of the relative rate of metabolism of a specific congener in a given species. *In vivo* disposition data were also obtained at exposures that were associated with induction of cytochromes P-4501A1 and 1A2 and other potentially adverse responses that could alter metabolism and disposition. Therefore, it may not be appropriate to directly extrapolate these data to predict the pharmacokinetics at low levels of exposure. Low dose extrapolations can be assisted by assessments of the potential for autoinduction of metabolism which may occur at exposures which are associated with enzyme induction. Characterization of the dose dependent disposition of 2,3,7,8-TCDD and related compounds is particularly important in the extrapolation of high exposure animal data to low exposure human data.

The excretion of metabolites of 2,3,7,8-TCDD and related compounds into bile represents a direct means for estimating the rate of metabolism, since biliary elimination depends on metabolism and is the major route for excretion of these compounds. The rate of metabolism of CDFs was estimated from the relative abundance of metabolites in rat bile (Poiger et al., 1989). The rate of biotransformation of 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF and 1,2,3,6,7,8-HxCDF were characterized as fairly high, moderate, low and very low, respectively. Kedderis et al. (1991b) observed 10% of the dose of [^{3}H]-2,3,7,8-TBDD excreted in bile 5 hours after intravenous administration of 1 nmol/kg to

male Fischer 344 rats. All biliary radioactivity was attributable to metabolites. This rate of elimination is similar to the fecal excretion (~8% of the dose) 24 hours after intravenous administration of 1 nmol/kg [^{3}H]-2,3,7,8-TBDD (Kedderis et al., 1991a) and reflects the effect of intravenous bolus versus oral administration on distribution and elimination. The large % dose excreted within the first few days may also be due to a rapidly excreted impurity in the radiolabeled 2,3,7,8-TBDD (Kedderis et al., 1992a). To assess the ability of 2,3,7,8-TCDD and 2,3,7,8-TBDD to induce their own metabolism (biliary elimination), rats were pretreated with 100 nmol/kg, *per os*, of each compound 3 days prior to intravenous injection of 1 nmol/kg of the respective [^{3}H] congeners. Biliary excretion of the radiolabeled dose was quantitatively and qualitatively unaffected by pretreatment, despite a 2-fold increase in hepatic levels of [^{3}H] in the pretreated animals and significant induction of cytochrome P-4501A1 and 1A2 (Kedderis et al., 1991b). Therefore, under these conditions, autoinduction of 2,3,7,8-TCDD and 2,3,7,8-TBDD metabolism did not occur in the rat *in vivo* at doses that elicited enhanced hepatic uptake. Similarly, Curtis et al. (1990) observed no change, or even an apparent decrease, in gastrointestinal contents and fecal elimination of TCDD equivalents in pretreated versus naive mice 24 hours after oral administration of [^{14}C]-2,3,7,8-TCDD, despite significantly enhanced levels of 2,3,7,8-TCDD in the livers of pretreated mice.

While the above studies suggest that autoinduction of 2,3,7,8-TCDD metabolism does not occur, other results indicate that metabolism may be induced under certain conditions. Poiger and Buser (1984) observed a small yet significant increase in biliary excretion over a 72-hour period, with pretreated rats (10 μg/kg, intraperitoneal) excreting 9.7±1.9% of the radiolabeled dose of 2,3,7,8-TCDD (200-300 μg/kg, *per os*) compared to 7.0±0.9% excreted by naive animals. In addition to being small changes, these results were obtained using a dose of 2,3,7,8-TCDD in excess of the LD_{50} in the rat. Poiger and Schlatter (1985) examined the influence of pretreatment with phenobarbital and 2,3,7,8-TCDD on the biliary excretion of [^{3}H]-2,3,7,8-TCDD metabolites in a dog given a single oral dose of the [^{3}H] congener (31 or 33.8 ng/kg). Without pretreatment, 24.5% of the

absorbed dose was excreted in the bile within 110 hours. Phenobarbital did not alter this rate, whereas pretreatment with 2,3,7,8-TCDD (10 μg/kg) 9 days earlier resulted in a doubling of the amount of metabolites excreted in bile (47.4%). Although this observation is limited to one dog and requires further investigation, the results suggest that significant autoinduction of 2,3,7,8-TCDD metabolism and biliary excretion may occur in the dog. Nonetheless, the small increase in metabolism and biliary excretion of 2,3,7,8-TCDD in the rat observed by Poiger and Buser (1984) and the negative results of Kedderis et al. (1991b) and Curtis et al. (1990) suggest that autoinduction of 2,3,7,8-TCDD metabolism and biliary excretion in the rat may not occur, or occurs to an extent that is not biologically relevant.

Limited data suggest that autoinduction of metabolism and biliary excretion does occur for CDFs in contrast to CDDs and BDDs. Pretreatment of rats with 2,3,7,8-TCDF (1.0 μmol/kg, 3 days earlier) significantly increased the biliary excretion of a subsequent dose of [^{14}C]-2,3,7,8-TCDF (McKinley et al., 1991). The naive rats excreted 5.69±2.35% of the dose over the initial 8 hours, while the pretreated rats excreted 13.18±3.15% of the [^{14}C]-2,3,7,8-TCDF. Similarly, pretreatment of rats with 2,3,4,7,8-PeCDF (500 μg/kg, *per os*, 3 days earlier) resulted in a 2-fold increase in the biliary elimination of a subsequent dose of [^{14}C]-2,3,4,7,8-PeCDF (Brewster and Birnbaum, 1987). These results suggest that pretreatment with 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF induces the metabolism of these congeners.

3,3',4,4'-TCB and 3,3',4,4'-TBB appear to be metabolized by a 3-methylcholanthrene-inducible form of hepatic cytochrome P-450 (1A1 and/or 1A2), which is also induced by 3,3',4,4'-TCB (Shimada and Sawabe, 1983; Mills et al., 1985). This suggests that these compounds can induce their own rate of metabolism and subsequent excretion.

Isolated hepatocytes in suspension culture have been demonstrated to provide a useful *in vitro* system for studying the hepatic metabolism of 2,3,7,8-TCDD under the same conditions in species that have a wide range of sensitivity to the compound (Olson et al., 1981). The *in vitro* rate of metabolism of 2,3,7,8-TCDD in guinea pig, rat, C57BL/6J mouse, DBA/2J mouse and hamster hepatocytes was

estimated to be 0.2, 1.2, 1.1, 0.9 and 1.2 pmol/mg protein/hour, respectively (Wroblewski and Olson, 1985, 1988; Shen and Olson, 1987). These results indicate that 2,3,7,8-TCDD is metabolized by the guinea pig liver at a rate ~5-fold less than that observed for the rat, mouse and hamster. The limited ability of the guinea pig to metabolize 2,3,7,8-TCDD can explain the limited excretion of 2,3,7,8-TCDD metabolites in feces, which represents the major route for 2,3,7,8-TCDD excretion (Olson, 1986). In addition, the limited metabolism in the guinea pig may partly explain the relatively long excretion half-life for 2,3,7,8-TCDD in the guinea pig and may contribute to the remarkable sensitivity of the guinea pig to the acute toxicity of this agent (Olson, 1986).

Isolated hepatocytes in suspension culture have been used as an *in vitro* system for studying the autoinduction of metabolism of 2,3,7,8-TCDD and related compounds. Wroblewski and Olson (1988) investigated the metabolism of [^{14}C]-2,3,7,8-TCDD (2.2 μM) in hepatocytes isolated from untreated 2,3,7,8-TCDD-, 3-MC-, isosafrole- and phenobarbital-pretreated rats and hamsters. In both species, 2,3,7,8-TCDD and 3-MC pretreatments elevated the rate of 2,3,7,8-TCDD metabolism by 5- to 6-fold, while phenobarbital pretreatment had no effect. Isosafrole produced a 1.8- to 2.5-fold increase in metabolism. These *in vitro* results at a high substrate concentration (2.2 μM) indicate that 2,3,7,8-TCDD can induce its own rate of metabolism in the rat and hamster. In contrast, 2,3,7,8-TCDD was not able to induce its own rate of metabolism in guinea pig and mouse hepatocytes (Wroblewski and Olson, 1985; Shen and Olson, 1987). Together, these results indicate that 2,3,7,8-TCDD is metabolized in the liver by a 2,3,7,8-TCDD inducible enzyme, which is expressed in the rat and hamster, but not in the guinea pig and mouse. More recently, the kinetics of 2,3,7,8-TCDD metabolism was investigated in isolated rat hepatocytes incubated with [^{3}H]-2,3,7,8-TCDD at concentrations of 0.01, 0.1 and 1.0 μM (Olson et al., 1991). Lower 2,3,7,8-TCDD concentrations in the media result in concentrations in hepatocytes which are more similar to the levels in the liver after *in vivo* exposure. For example, the concentration of 2,3,7,8-TCDD in hepatocytes incubated at 0.01 μM are similar to hepatic levels after *in vivo* exposure of rats at a dose of ~10 μg/kg. At 0.01

and 0.1 μM, the rate of metabolism of [^{3}H]-2,3,7,8-TCDD was similar in hepatocytes isolated from control and 2,3,7,8-TCDD pretreated rats, while at 1.0 μM, 2,3,7,8-TCDD metabolism was greater in hepatocytes isolated from 2,3,7,8-TCDD pretreated rats. The results indicate that 2,3,7,8-TCDD can induce its own rate of metabolism in the rat, but only at high hepatic concentrations, which are generally not attained after *in vivo* exposure. A dose-dependent autoinduction of 2,3,7,8-TCDD metabolism is consistent with the lack of autoinduction of 2,3,7,8-TCDD metabolism and biliary excretion in the rat (Kedderis et al., 1991b; Curtis et al., 1990).

The metabolism of [^{3}H]-2,3,7,8-TCDF was also investigated in isolated rat hepatocytes incubated at concentrations of 0.01, 0.1 and 1.0 μM (Olson et al., 1991). At all concentrations, hepatocytes from 2,3,7,8-TCDD pretreated rats metabolized 2,3,7,8-TCDF at a rate from 4- to 25-fold greater than that observed in hepatocytes from control rats. The results indicate that 2,3,7,8-TCDF is metabolized in rat liver by a 2,3,7,8-TCDD inducible enzyme, possibly cytochrome P-4501A1 or 1A2. These *in vitro* results support the *in vivo* autoinduction of 2,3,7,8-TCDF metabolism and biliary elimination observed in the rat (McKinley et al., 1991).

There is *in vivo* and *in vitro* data suggesting that autoinduction of 2,3,7,8-TCDD and 2,3,7,8-TBDD metabolism does not occur in the rat after exposure to sublethal doses of these agents. This is in contrast to 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF where *in vivo* and *in vitro* results support the autoinduction of metabolism and biliary elimination of these compounds in the rat.

1.3.4. Excretion in Animals. Data regarding the excretion of 2,3,4,7-TCDD and related compounds after exposure to a single radiolabeled congener (see Table 1-8) support the assumption of a first-order elimination process consisting of one or more components. These studies show that 2,3,7,8-TCDD was excreted slowly from all species tested, with half-lives ranging from 11 days in the hamster to 2120 days in humans. 2,3,7,8-TCDD is exceptionally persistent in humans relative to other animal models. Elimination data in tissues (see Tables 1-5 and 1-6) also indicate that 2,3,7,8-TCDD and related compounds are exceptionally persistent in nonhuman primates (Bowman et al., 1989; Neubert et al., 1990). These

differences may also be in part related to the dose-dependency of the excretion of these compounds. In general, the congener and species specific rate of elimination of 2,3,7,8-TCDD and related compounds from major tissue depots (see Table 1-5) is similar to the excretion data summarized in Table 1-8.

In the Syrian Golden hamster, the mammalian species least sensitive to the acute toxicity of 2,3,7,8-TCDD, excretion occurred readily through both the urine (35% of administered dose, 41% of total excreted radioactivity) and feces (50% of the administered dose, 59% of total excreted radioactivity) (Olson et al., 1980b). A similar excretion pattern was observed in mice, although there was significant strain variability (Gasiewicz et al., 1983; Birnbaum, 1986). In all the other species, excretion occurred mainly through the feces, with relatively minor amounts of 2,3,7,8-TCDD metabolites found in the urine (Piper et al., 1973; Allen et al., 1975; Olson, 1986; Rose et al., 1976; Gasiewicz and Neal, 1979; Pohjanvirta et al., 1990). Results in Table 1-8 also indicate that fecal elimination was the primary route for the excretion of 1,2,3,7,8-PeCDD, OCDD, 2,3,7,8-TBDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, and 3,3',4,4'-TCB. Only Piper et al. (1973) reported the excretion of metabolites in the expired air. During 21 days following administration of a single oral dose of [^{14}C]-2,3,4,7-TCDD to rats, 3.2% of the administered radioactivity (4.6% of the excreted radioactivity) was recovered in the expired air.

Studies in the rat, guinea pig, hamster and mouse have found that essentially all of the 2,3,7,8-TCDD-derived radioactivity excreted in the urine and bile corresponds to metabolites of 2,3,7,8-TCDD (see Table 1-8). The apparent absence of 2,3,7,8-TCDD metabolites in liver and fat suggests that once formed, the metabolites of 2,3,7,8-TCDD are excreted readily. Thus, urinary and biliary elimination of 2,3,7,8-TCDD depends on metabolism of the toxin. The more limited data for other compounds also suggest that this relationship may be true for 1,2,3,7,8-PeCDD, 2,3,7,8-TBDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, and 3,3',4,4'-TCB (see Table 1-8).

Although urine and bile appear to be free of unmetabolized 2,3,7,8-TCDD, data indicate that 2,3,7,8-TCDD and its metabolites are excreted in the feces of guinea pigs, rats, mice and hamsters treated with [^{3}H]- and/or [^{14}C]-2,3,7,8-TCDD

(see Table 1-8). While 15-35% of the 2,3,7,8-TCDD-derived radioactivity in rat, mouse and hamster feces represents unchanged 2,3,7,8-TCDD, 81% of the radioactivity in guinea pig feces represents unmetabolized 2,3,7,8-TCDD (Olson, 1986; Neal et al., 1982; Gasiewicz et al., 1983; Olson et al., 1980). The daily presence of unchanged 2,3,7,8-TCDD in feces and its absence in bile suggests that direct intestinal elimination may be the source for the fecal excretion of 2,3,7,8-TCDD. Data also suggest that direct intestinal elimination of parent compound contributes to the fecal excretion for 2,3,7,8-TBDD (Kedderis et al., 1991). While the direct intestinal elimination of parent compound may occur for other congeners (see Table 1-8), this conclusion cannot be made at this time due to the lack of experimental data. Nonetheless, the species-specific fecal excretion of 2,3,7,8-TCDF is very similar to that observed for 2,3,7,8-TCDD, with >90% of the 2,3,7,8-TCDF-derived radioactivity excreted in guinea pig feces representing parent compound (Decad et al., 1981a). In addition, the excretion of unchanged CDDs and CDFs was detected in rat feces after subcutaneous exposure to a defined mixture of congeners (Abraham et al., 1989). Studies in lactating rats have also found that unchanged 2,3,7,8-TCDD may be excreted in the milk of lactating animals (Moore et al., 1976; Lucier et al., 1975; Nau et al., 1986). Lactation, direct intestinal elimination, and perhaps sebum may serve as routes for excretion of 2,3,7,8-TCDD, which do not depend on metabolism of the toxin. These data suggest that the *in vivo* half-life for elimination of 2,3,7,8-TCDD and related compounds only provides an approximation of the rate of metabolism of these compounds in a given animal. The results in Table 1-8 do suggest that 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, and 3,3',4,4'-TCB are metabolized and excreted more rapidly that 2,3,7,8-TCDD, 2,3,7,8-TBDD, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF and OCDD.

The rate of excretion of 2,3,7,8-TCDD and related compounds is species- and congener-specific (see Table 1-8). 2,3,7,8-TCDD is most persistent in human and nonhuman primates. In the hamster, the least sensitive species to the acute toxicity of 2,3,7,8-TCDD, the mean $t_{1/2}$ was 10.8 days (Olson et al., 1980a,b), and in the guinea pig, the most sensitive species to the acute toxicity of 2,3,7,8-

TCDD, the mean $t_{1/2}$ was 94 days (Olson, 1986). 2,3,7,8-TCDF was also most persistent in the guinea pig, with a $t_{1/2}$ of 20-40 days (Decad et al., 1981a; Ioannou et al., 1983). Furthermore, results indicate that the relatively limited ability of the guinea pig to metabolize 2,3,7,8-TCDD and -TCDF may contribute to the greater persistence and greater acute toxicity of these congeners in the guinea pig.

The time distribution, metabolism and excretion of 2,3,7,8-TCDD were also investigated in Han/Wistar and Long-Evans rats, which were, respectively, more resistent (LD_{50}>3000 μg/kg) versus more susceptible (LD_{50} ~10 μg/kg) to the acute toxicity of 2,3,7,8-TCDD (Pohjanvirta et al., 1990). The results suggest that the metabolism and disposition of 2,3,7,8-TCDD do not have a major role in explaining the strain differences in toxicity.

The intraspecies differences in the $t_{1/2}$ of 2,3,7,8-TCDD in three mouse strains may be due to the finding that the DBA/2J strain possesses ~2-fold greater adipose tissue stores than the C57BL/6J and B6D2F_1/J strains (Gasiewicz et al., 1983b). The sequestering of the lipophilic toxin in adipose tissue stores of the DBA/2J mouse may contribute to the greater persistence of 2,3,7,8-TCDD in this strain. Birnbaum (1986) examined the effect of genetic background on the distribution and excretion of 2,3,7,8-TCDD in two sets of congenic mouse strains in which the congenic pairs differed only at the Ah locus. The Ah locus had no effect on the tissue distribution or excretion of 2,3,7,8-TCDD. Thus, the distribution and excretion of 2,3,7,8-TCDD were primarily governed by the total genetic background rather than the allele present at the Ah locus. These findings are consistent with the *in vitro* results of Shen and Olson (1987), who found that the hepatic uptake and metabolism of 2,3,7,8-TCDD do not correlate with genetic differences at the murine Ah locus. However, it is important to note that all of these are relatively high-dose studies, which may not allow for detection of Ah receptor-mediated effects on disposition.

Although the dose-related tissue distribution of 2,3,7,8-TCDD and related compounds has been described recently, very limited data are available on the dose-related excretion of these compounds. Rose et al. (1976) investigated the

elimination of [^{14}C]-2,3,7,8-TCDD in rats given repeated oral doses of 0.01, 0.1 or 1.0 μg/kg/day Monday through Friday for 7 weeks or a single dose of 1.0 μg/kg. In the single-dose study, no ^{14}C was excreted in the urine or expired air; in the repeated-dose study, however, 3-18% of the cumulative dose was excreted in the urine by 7 weeks. This study indicated that steady-state concentrations will be reached in the bodies of rats in ~13 weeks. The rate constant defining the approach to steady-state concentrations was independent of the dose of 2,3,7,8-TCDD over the range studied. Relatively small changes in the excretion of 2,3,7,8-TBDD were also observed after exposures at 1 and 100 nmol/kg (Kedderis et al., 1991). These results are consistent with the *in vivo* and *in vitro* evidence suggesting that autoinduction of 2,3,7,8-TCDD and 2,3,7,8-TBDD metabolism does not occur in the rat after exposure to sublethal doses of these compounds (Kedderis et al., 1991b; Curtis et al., 1990; Olson et al., 1991). In contrast to these compounds, 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF can induce their own rate of metabolism and biliary excretion (Brewster and Birnbaum, 1987; McKinley et al., 1991; Olson et al., 1991). Autoinduction of metabolism would suggest that these compounds may exhibit dose-related excretion, with longer half-lives for elimination at lower doses, which are not associated with enzyme induction. Further data are needed to test this hypothesis.

1.3.5. Excretion in Humans. Poiger and Schlatter (1986) investigated the excretion of 2,3,7,8-TCDD in a 42-year-old man (92 kg) after ingestion of 105 ng (1.14 ng/kg) [^{3}H]-2,3,7,8-TCDD in 6 mL corn oil (see Table 1-8). The half-life for elimination was estimated to be 2120 days. Table 1-9 summarizes additional half-life estimates for 2,3,7,8-TCDD and related compounds in humans, based on serum and/or adipose tissue concentrations at two or more time points. In another study, the half-life of 2,3,7,8-TCDD in humans was estimated to be ~7 years on the basis of 2,3,7,8-TCDD levels in serum samples taken in 1982 and 1987 from 36 of the Ranch Hand personnel who had 2,3,7,8-TCDD levels >10 ppt in 1987 (Pirkle et al., 1989). These studies indicate that 2,3,7,8-TCDD is exceedingly persistent in humans. Estimated half-lives for other congeners in Table 1-9 range from 0.8-10 years. The half-life values in Table 1-9 are rough estimates based on a small number of individuals and based on analysis at as few as two

TABLE 1-9

Half-Life Estimates for 2,3,7,8-TCDD and Related Compound in Humans

Chemical	Exposure Incident	Number of Individuals	Sample	Time Period Between First and Last Analysis	Number of Time Points	Half-Life (years)	Reference
CDDs							
2,3,7,8-TCDD	Ranch Hand Vietnam veterans	36	serum	5 years	2	7.1[a]	Pirkle et al., 1989
1,2,3,6,7,8-HxCDD	technical pentachlorophenol in wood of home	1	adipose tissue	28 months	2	3.5	Gorski et al., 1984
1,2,3,4,6,7,8-HpCDD	technical pentachlorophenol in wood of home	1	adipose tissue	28 months	2	3.2	Gorski et al., 1984
OCDD	technical pentachlorophenol in wood of home	1	adipose tissue	28 months	2	5.7	Gorski et al., 1984
CDFs							
2,3,4,7,8-PeCDF	Binghamton, New York, state office building	1	adipose tissue blood combined	initial 43 months final 29 months total 6 years	4 4 7	4.7 7.2 4.5	Schecter et al., 1990
1,2,3,4,7,8-HxCDF	Binghamton, New York, state office building	1	adipose tissue blood combined	initial 43 months final 29 months total 6 years	4 4 7	2.9 4.4 4.0	Schecter et al., 1990
1,2,3,6,7,8-HxCDF	Binghamton, New York, state office building	1	adipose tissue blood combined	initial 43 months final 29 months total 6 years	4 4 7	3.5 4.3 4.9	Schecter et al., 1990
1,2,3,4,6,7,8-HpCDF	Binghamton, New York, state office building	1	adipose tissue blood combined	initial 43 months final 29 months total 6 years	4 4 7	6.5 4.1 6.8	Schecter et al., 1990
2,3,4,7,8-PeCDF	Yu-Cheng	4 3 2	blood	initial 2.9 years final 2.7 years total 5.6 years	2 2 3	1.3 2.9 1.7	Ryan, 1989
1,2,3,4,7,8-HxCDF	Yu-Cheng	4 3 2	blood	initial 2.9 years final 2.7 years total 5.6 years	2 2 3	2.1 5.1 2.4	Ryan, 1989
1,2,3,4,6,7,8-HpCDF	Yu-Cheng	4 3 2	blood	initial 2.9 years final 2.7 years total 5.6 years	2 2 3	1.6 6.1 2.4	Ryan, 1989

TABLE 1-9 (cont.)

Chemical	Exposure Incident	Number of Individuals	Sample	Time Period Between First and Last Analysis	Number of Time Points	Half-Life (years)	Reference
2,3,4,7,8-PeCDF 1,2,3,4,7,8-HxCDF 1,2,3,4,6,7,8-HpCDF	Yu-Cheng	3	blood	9 years	5-6	2-3	Ryan and Masuda, 1991
2,3,4,7,8-PeCDF 1,2,3,4,7,8-HxCDF	Yusho	9	blood	7 years	3-5	>5	Ryan and Masuda, 1991
1,2,3,4,6,7,8-HpCDF	technical pentachlorophenol in wood of home	1	adipose tissue	28 months	2	<1.7	Gorski et al., 1984
OCDF	technical pentachlorophenol in wood of home	1	adipose tissue	28 months	2	1.8	Gorski et al., 1984
PCBs							
3,3',4,4',5-PeCB	Yu-Cheng	NA	blood	NA	NA	<1	Ryan and Masuda, 1991
3,3',4,4',5,5'-HxCB	Yu-Cheng	NA	blood	NA	NA	10	Ryan and Masuda, 1991

[a]95% confidence interval about the median of 5.8–9.6 years

NA = Not applicable

time points. Phillips (1989) discusses this issue. Estimates also assume a simple, single compartment, first-order elimination process. Recent data suggest a biphasic elimination of 2,3,7,8-TCDD, with a half-life >7 years (Birnbaum, 1992).

Ryan and Masuda (1991) reported on their continuing investigation into the elimination of CDFs in humans from the Yusho and Yu-Cheng rice oil poisonings. Yu-Cheng individuals had CDF blood levels on a lipid basis of 1-50 μg/kg, while Yusho patients had levels of 0.1-5 μg/kg. In the Yu-Cheng individuals, half-lives for three CDFs were 2-3 years, while elimination from Yusho individuals was more variable and slower, with half-lives >5 years (see Table 1-9) and, in several cases, no measurable elimination during the 7 years in which samples were available. The limited results suggest that clearance of these CDFs in the human is biphasic, with faster elimination at higher exposure. Schecter et al. (1990) and Ryan (1989) also reported longer half-life values for CDFs in humans at later time points after exposure, when concentrations are closer to the background levels of individuals with no unusual exposure.

Due to the lipophilic nature of milk, milk secretion can provide a relatively efficient mechanism for decreasing the body burden of 2,3,7,8-TCDD in females. As discussed by Graham et al. (1986), this elimination of 2,3,7,8-TCDD through mother's milk can result in high exposure levels in the infant. Since both milk and the fatty tissues of fish are essentially providing an oily vehicle, it would be likely that these sources would provide 2,3,7,8-TCDD in a form that is readily bioavailable.

Several investigators have quantified the levels of 2,3,7,8-TCDD in human milk samples. Many of the milk samples were pooled (Jensen, 1987). Rappe et al. (1984) reported levels of 1-3 ppt 2,3,7,8-TCDD in milk fat (lipid adjusted) from five volunteers in West Germany, and in a later report, Rappe et al. (1985) reported an average level of 0.6 ppt 2,3,7,8-TCDD in milk fat from four volunteers in northern Sweden. Furst et al. (1986) reported an average level of 9.7 ppt 2,3,7,8-TCDD in milk fat from three individuals in the Netherlands and <1.0 ppt 2,3,7,8-TCDD in milk fat from two individuals in Yugoslavia. Nygren et al. (1986) reported average levels of 2,3,7,8-TCDD in human milk samples from four subjects in Sweden to be 0.6 ppt in milk fat, in five subjects from West

Germany to be 1.9 ppt in milk fat, and in four subjects from Vietnam to be <0.5 ppt in milk fat.

High levels of 2,3,7,8-TCDD have been detected in the milk of mothers exposed to high levels of 2,3,7,8-TCDD in the environment. Reggiani et al. (1980) reported levels between 2.3 and 28.0 ppt 2,3,7,8-TCDD in whole milk from mothers in Seveso. Baughman (1975) reported levels between 40.0 and 50.0 ppt 2,3,7,8-TCDD in whole milk from mothers in South Vietnam. Schecter et al. (1987) also found high ppt levels of 2,3,7,8-TCDD in human milk samples from South Vietnam. These authors found that samples taken in 1985 from South Vietnamese mothers were comparable to the level of 2,3,7,8-TCDD presently found in North American human milk samples (5 ppt).

1.4. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

PB-PK models have been developed for 2,3,7,8-TCDD in C57BL/6J and DBA/2J mice (Leung et al., 1988), rats (Leung et al., 1990) and humans (Kissel and Robarge, 1988). PB-Pk models incorporate known or estimated anatomical, physiological and physicochemical parameters to describe quantitatively the disposition of a chemical in a given species. PB-Pk models can assist in the extrapolation of high-to-low dose kinetics within a species, estimating exposures by different routes of administration, calculating effective doses and extrapolating these values across species (Scheuplein et al., 1990). Table 1-10 summarizes the pharmacokinetic parameters for 2,3,7,8-TCDD that were used in developing these PB-Pk models. In many cases, these parameters were estimated from *in vivo* experimental data.

A five-compartment (blood, liver, fat, muscle/skin, viscera), flow-limited PB-Pk model for 2,3,7,8-TCDD was developed for the Ah-responsive C57BL/6J mouse and the Ah-nonresponsive DBA/2J mouse (Leung et al., 1988). The model also included binding in the hepatic cytosol and hepatic microsomes and first-order hepatic metabolism. There was general agreement between the simulated description generated by the model and the experimental disposition data of Gasiewicz et al. (1983). The greater accumulation of 2,3,7,8-TCDD in the liver of the C57BL/6J mouse, compared to the DBA/2J mouse, was not attributable to the 2-fold greater total fat content in the DBA/2J strain. The authors suggested that strain difference in hepatic disposition was due to differing affinity of

TABLE 1-10

Pharmacokinetic Parameters for 2,3,7,8-TCDD Used in PB-Pk Models

	C57BL/6J Mouse[a]	DBA/2J Mouse[a]	Sprague-Dawley Rat[b]	Human[c]	Female C57BL/6J Mice[d]	
					Naive	Pretreated
PARTITION COEFFICIENT (TISSUE/BLOOD)						
Liver	20	20	20	25	10	10
Fat	350	350	350	300	300	300
Richly perfused (kidney)	20	20	20	7-10	10	10
Slowly perfused (skin)	250	250	40	30	200	200
Slowly perfused (muscle)	250	250	40	4	3	3
BIOCHEMICAL CONSTANTS						
Binding capacity to hepatic cytosolic protein (nmol/liver)	0.0042	0.0042	0.054	-	0.0042	0
Binding affinity to hepatic cytosolic protein (nM)	0.29	2	0.015	-	0.29	0.29
Binding capacity to hepatic microsomal protein (nmol/liver)	20	20	-	-	1.75	20
Noninduced binding capacity to hepatic microsomal protein (nmol/liver)	-	-	25	-	-	-
Induced binding capacity to hepatic microsomal protein (μmol/liver)	-	-	175	-	-	-
Binding affinity to hepatic microsomal protein (nM)	20	75	7	-	20	20
First-order metabolic rate constant (per hour per kg liver)	3.25	1.75	2.0	-	1.0	3.0
Absorption constant from gastrointestinal tract into liver (per hour)	0.02	0.02	0.2	-	0.04	0.15
Binding to blood	2.5	2.5	2.5	-	1.0	3.0

[a]Leung et al. (1988)

[b]Leung et al. (1990)

[c]Kissel and Robarge (1988)

[d]Leung et al. (1990) modeled the disposition of [^{125}I]-2-iodo-3,7,8-TCDD, an analog of 2,3,7,8-TCDD, following a single exposure (0.1 nmol/kg) in naive female C57BL/6J mice and in mice pretreated 3 days earlier with an inducing dose of 2,3,7,8-TCDD (0.1 μmol/kg).

2,3,7,8-TCDD for the microsomal binding protein in the two strains, and proposed using a different microsomal dissociation constant for each strain. Alternatively, the strain difference in hepatic disposition may be due to the different doses of 2,3,7,8-TCDD needed to induce cytochrome P-4501A2 in the two strains. In contrast to the high capacity/low affinity hepatic microsomal binding proteins, the low capacity/high affinity hepatic cytosolic binding protein (Ah receptor) did not play a major role in determining the overall tissue distribution pattern of 2,3,7,8-TCDD in this model.

A similar five-compartment PB-Pk model was developed to describe the tissue disposition of 2,3,7 8-TCDD in the Sprague-Dawley rat (Leung et al., 1990). This description included blood, liver (cytosolic receptor and microsomal binding protein), fat, muscle/skin and visceral tissue compartments. The authors found generally good agreement between the PB-Pk model simulated data and the experimental data for the single-dose study of Rose et al. (1976) and the 7- and 13-week multiple-dose studies of Kociba et al. (1978). The model was not satisfactory for the 2-year feeding study of Kociba et al. (1978), underpredicting the 2,3,7,8-TCDD concentration in the fat at the low dose (0.001 μg/kg/day) and overestimating the concentration achieved at the high dose (0.1 μg/kg/day). The model found the hepatic disposition of 2,3,7,8-TCDD to be dependent on the high capacity/low affinity hepatic microsomal binding protein with a dissociation constant of 7 nM and a basal and induced concentration in the liver of 25 and 175 nmol/liver, respectively. As discussed earlier, Voorman and Aust (1987, 1989) and Poland et al. (1989a,b) provided evidence that this binding species is cytochrome P-4501A2. Induction of the microsomal binding protein was necessary in order to account for the differences in hepatic disposition at low (0.01 μg/kg) and high (1.0 μg/kg) daily doses of 2,3,7,8-TCDD. The dose-dependent tissue distribution of 2,3,7,8-TCDD was also discussed earlier. As in the mouse PB-Pk model (Leung et al., 1988), the low capacity/high affinity hepatic cytosolic binding protein (Ah receptor) was not a major factor in directly influencing the hepatic disposition of 2,3,7,8-TCDD. The dissociation constant of the cytosolic Ah receptor *in vivo* was estimated to be 15 pM by fitting enzyme induction data from McConnell et al. (1984).

A PB-Pk model was also developed for female C57BL/6J mice for [^{125}I]-2-iodo-3,7,8-TCDD, an analog of 2,3,7,8-TCDD (Leung et al., 1990). Mice were pretreated with 0.1 μmol/kg of 2,3,7,8-TCDD or the vehicle only, followed by 2-iodo-3,7,8-TCDD (0.1 nmol/kg) 3 days later. Naive mice had liver/fat 2-iodo-3,7,8-TCDD concentration ratios of 0.17-0.38, while the 2,3,7,8-TCDD pretreated mice had ratios of 2.0-6.1. This is in agreement with the dose-dependent tissue distribution of 2,3,7,8-TCDD described earlier (Abraham et al., 1988; Poiger et al., 1989). As with 2,3,7,8-TCDD, the model found that the 2-iodo-3,7,8-TCDD concentration in the liver was most sensitive to the binding capacity of the hepatic microsomal protein. Whole-body elimination of 2-iodo-3,7,8-TCDD approximated first-order kinetics, and induction by pretreatment with an inducing dose of 2,3,7,8-TCDD almost doubled the rate of excretion ($t_{1/2}$ of 14.2 days in naive versus 8.0 days in induced mice) (see Table 1-8). The distribution in naive and pretreated mice was described by a PB-Pk model in which induction (2,3,7,8-TCDD pretreatment) increased the amount of hepatic microsomal binding protein from 1.75-20 nmol/liver and increased the rate constant for metabolism of free 2-iodo-3,7,8-TCDD from 1-3 hours/kg liver. Although the more rapid elimination of 2-iodo-3,7,8-TCDD in 2,3,7,8-TCDD pretreated mice suggests that the rate of metabolism of 2-iodo-3,7,8-TCDD was induced by pretreatment, no data were provided on the effect of this pretreatment of body weight and composition, which may in turn alter the rate of elimination. 2,3,7,8-TCDD pretreatment may also alter deiodinase activity. Furthermore, *in vivo* and *in vitro* studies suggest that the autoinduction of 2,3,7,8-TCDD metabolism may not occur under these conditions. Kedderis et al. (1991b) and Curtis et al. (1990) found no autoinduction of 2,3,7,8-TCDD metabolism and biliary excretion in the rat. In addition, Shen and Olson (1987) found that while 2,3,7,8-TCDD pretreatment of C57BL/6J mice increased the uptake of 2,3,7,8-TCDD by hepatocytes in suspension culture, pretreatment did not increase the rate of metabolism of 2,3,7,8-TCDD by hepatocytes. Therefore, this PB-Pk model may not accurately describe the metabolism of 2,3,7,8-TCDD for exposures which result in varying degrees of induction of the hepatic monooxygenase system. While the dose-dependent pharmacokinetics of 2,3,7,8-TCDD may not include autoinduction of 2,3,7,8-TCDD

metabolism, this may not be the case for other CDDs, BDDs, CDFs, BDFs, PCBs and PBBs. For example, the autoinduction of metabolism has been reported for CDFs (Brewster and Birnbaum, 1987; McKinley et al., 1991; Olson et al., 1991).

Andersen et al. (1992) recently derived a receptor-mediated PB-PK model for the tissue distribution and enzyme inducing properties of 2,3,7,8-TCDD. The data used for this analysis were from two previously published studies with Wistar rats (Abraham et al., 1988; Krowke et al., 1989). The model was used to examine the tissue disposition of 2,3,7,8-TCDD and the induction of both a dioxin-binding protein (presumably cytochrome P-4501A2) and cytochrome P-4501A1.

Kohn et al. (1992) recently developed a mechanistic model of the effects of dioxin on glue expression in the rat liver (referred to as the NIEHS model). The model includes the tissue distribution of 2,3,7,8-TCDD in the rat and its effect on the concentrations of CYP1A1 and CYP1A2, and the effects of 2,3,7,8-TCDD on the Ah, estrogen and EGF receptors over a wide 2,3,7,8-TCDD dose range. Experimental data from Tritscher et al. (1992) and Sewall et al. (1992) were incorporated into the NIEHS model. Female Sprague-Dawley rats were injected with an initiating dose of diethylnitrosamine, and after 20 days, the rats were exposed biweekly to 2,3,7,8-TCDD in corn oil by gavage at doses equivalent to 3.5-125 ng/kg/day for 30 weeks. The NIEHS model predicts a linear relationship between administered dose and the concentration in the liver over this dose range, which is in agreement with the data of Tritscher et al. (1992). The biochemical response curves for all these proteins were hyperbolic, indicating a proportional relationship between target tissue dose and protein concentration at low administered doses of 2,3,7,8-TDCDD.

A fugacity-based PB-Pk model for the elimination of 2,3,7,8-TCDD from humans was developed by Kissel and Robarge (1988). Transport within the body was assumed to be perfusion-limited (flow-limited). 2,3,7,8-TCDD was assumed to be uniformly distributed within each tissue or fluid phase, and tissue levels were considered to be in equilibrium with exiting fluids (blood, bile, urine). 2,3,7,8-TCDD appears to be poorly metabolized in humans, thus reducing the necessity of modeling the fate of metabolites. 2,3,7,8-TCDD also seems to exhibit fugacity-based partitioning behavior in humans as evidenced by relatively constant lipid-based tissue distribution (Leung et al., 1990; Ryan et al., 1987),

although this is not the case in rodents (Leung et al., 1988, 1990). With a daily human background intake of 2,3,7,8-TCDD in North America of ~50 pg/day (Travis and Hattemer-Frey, 1987), the steady state adipose tissue concentration predicted by the model, assuming no metabolism, was 7.7 ppt. This is similar to the lipid-based blood tissue levels reported in the general population with no known unusual exposure. The model was also used to predict the elimination of 2,3,7,8-TCDD from Ranch Hand Vietnam veterans. The model simulation 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 adipose tissue concentrations of 100, 50, 30, 20, 15 and 10 ppt, respectively. The model predicted half-lives are similar to the experimental value of 7.1 years, based on analysis of 2,3,7,8-TCDD in blood lipids of veterans with adipose burdens greater than 10 ppt (Pirkle et al., 1989) (see Table 1-9). The apparent half-lives derived from the model increased as the adipose tissue concentrations approached the steady-state level associated with background exposure. Ryan and Masuda (1991) also reported a similar relationship for CDFs, with experimentally derived half-lives increasing in individuals with lower body burdens of the compounds. Finally, the model was also found to approximate the elimination of 2,3,7,8-TCDD from one volunteer as reported by Poiger and Schlatter (1986). Taken together, the comparisons described above suggest that a fugacity-based PB-Pk model for 2,3,7,8-TCDD in humans can provide one method for describing the elimination of 2,3,7,8-TCDD from humans.

Kedderis et al. (1992a,b) recently developed a PB-PK model for 2,3,7,8-TBDD in the rat. The model is based on previously developed physiologically-based models for 2,3,7,8-TCDD (Leung et al., 1990; Poland et al., 1989) and utilizes published data on the disposition of a single exposure to 2,3,7,8-TBDD at a dose of 1 or 100 nmol/kg, intravenous (Kedderis et al., 1991a,b) and dermal disposition data (Jackson et al., 1991). In the model, the dose- and time-dependent accumulation in the liver was attributed to specific binding of 2,3,7,8-TBDD with the inducible protein, CYP1A2. The model also includes diffusion-limited tissue uptake of 2,3,7,8-TBDD, transluminal excretion of parent compound via the gut into the feces, growth of tissue compartments and a separate skin compartment. This model provides further validation of the model structure

originally developed to describe important dispositional determinants for 2,3,7,8-TCDD.

A five-compartment (blood, liver, fat, skin, muscle) flow-limited physiological model was developed to describe the tissue distribution and excretion of 2,3,7,8-TCDF-derived material in rats, mice and monkeys (King et al., 1983), based on experimental data reported earlier (Birnbaum et al., 1980, 1981; Decad et al., 1981b). Partition coefficients (tissue/blood distribution ratios) and metabolic clearances were estimated from *in vivo* experimental data and are summarized in Table 1-11. All pharmacokinetic parameters for 2,3,7,8-TCDF were based on *in vivo* data after a single intravenous exposure at a dose of 0.1 μmol/kg (30.6 μg/kg). Therefore, the model is limited in not considering the potential dose-related distribution and excretion of 2,3,7,8-TCDF. Recent studies indicate that 2,3,7,8-TCDF is able to induce its own rate of metabolism and biliary excretion at higher doses (McKinley et al., 1991; Olson et al., 1991). This model will need to be revised as additional data on the dose-related distribution and excretion of 2,3,7,8-TCDF become available.

PB-Pk models are primarily limited by the availability of congener and species-specific data that accurately describe the dose- and time-dependent disposition of 2,3,7,8-TCDD and related compounds. The pharmacokinetic parameters summarized in Tables 1-10 and 1-11 were derived from available *in vivo* and *in vitro* experimental data. As additional data become available, particularly on the dose-dependent disposition of these compounds, more accurate models can be developed. In developing a suitable model in the human, it is also important to consider that the half-life estimate of 7.1 years for 2,3,7,8-TCDD was based on two serum values taken 5 years apart, with the assumption of a single compartment, first-order elimination process (Pirkle et al., 1989). It is likely that the excretion of 2,3,7,8-TCDD in humans is more complex, involving several compartment, tissue-specific bonding proteins and a continuous daily background exposure. Furthermore, changes in body weight and body composition should also be considered in developing PB-Pk models for 2,3,7,8-TCDD and related compounds in humans.

An empirical model of dioxin (toxic equivalents) disposition in animals and humans has also been recently developed by Carrier and Brodeur (1991). The

TABLE 1-11

Pharmacokinetic Parameters for 2,3,7,8-TCDF Used in the PB-Pk Model Described by King et al. (1983)

	C57BL/6J Mouse	DBA/2J Mouse	Fischer 344 Rat	Rhesus Monkey
PARTITION COEFFICIENTS				
Liver	130	100	100	30
Fat	25	40	35	30
Skin	8	12	4	7
Muscle	2	4	2	2
CLEARANCES				
Metabolism	0.07	0.06	1.0	2.25
Km (mL/minute/kg)	2.8	2.4	4.0	0.45
Metabolism excretion ratio K_k/K_L[a]	0.14	0.27	0.03	0.19

[a]Urinary clearance/biliary clearance

kinetic analysis begins with the observation that the tissue distribution of dioxin-like HPAH in humans and in animals is dose-dependent (or, alternately, body-burden dependent). As total body burden of TCDD equivalents increases, the proportion of the body burden associated with the liver increases toward a maximum value. The data were then analyzed with an empirical, saturable binding isotherm equation:

$$liver\ fraction\ (f_H) = f_{max} C_{body} / (K_d + C_{body})$$

If the body burden—C_{body} (μg/kg)—is considered a surrogate for liver concentration, this equation can be loosely interpreted as the induction of binding species in the liver as dose increases. In the analyses, Kd was found to be very similar for people and experimental animals, indicating similar protein induction dynamics in various animal species. This model, however, is not physiologically-based and the terms, C_{body} and f_{max}, are difficult to interpret in biological terms. In working with different isomers, f_{max} and K_d values vary somewhat, presumably due to binding affinities in the liver.

This empirical model is successful in providing a description that "fits" the observed data in various species. It still is largely a fitting exercise to a particular equation, not an examination of biology by computer modeling. In addition, there are at least two assertions that seem incorrect. First, the assumption that the limit of the hepatic fraction at very low doses is zero. It seems more likely that the limit is some finite value, determined by liver partitioning of dioxin and the binding parameters of the Ah receptor, and the dioxin binding species in the liver in the linear, low-dose region. Secondly, that metabolism of dioxin becomes saturated with the maximum induction of liver sequestration of dioxin. There is no justification for this at present. Nevertheless, the model indicates clearly that with respect to dosimetry and induction of hepatic binding species for dioxin, people and rodents are very similar. Furthermore, the empirical model of Carrier and Brodeur (1991) is generally consistent with the PB-PK models.

1.5. PHARMACOKINETICS IN SPECIAL POPULATIONS

1.5.1. Pregnancy and Lactation (Prenatal and Postnatal Exposure of Offspring). The distribution and excretion of [^{14}C]-2,3,7,8-TCDD (30 μg/kg) and [^{14}C]-2,3,7,8-TCDF (800 μg/kg) were studied in pregnant C57BL/6N mice after oral exposure on gestation day 11 (Weber and Birnbaum, 1985). The distribution and excretion of 2,3,7,8-TCDD and 2,3,7,8-TCDF in pregnant mice were similar to that of males of the same strain (Gasiewicz et al., 1983; Decad et al., 1981b) (see Tables 1-5 and 1-8), although elimination rates were higher in the pregnant mice for both congeners. For 2,3,7,8-TCDD, liver, urinary and fecal elimination were 3.0, 3.4 and 14.4 times faster than that reported for males. For 2,3,7,8-TCDF, liver, urinary and fecal elimination were 1.3, 1.8 and 1.8 times faster than observed for males. Elimination data from pregnant mice was based on only three time points (gestation days 12, 13 and 14) and thus represents only rough estimates. In addition, the greater fecal excretion could have been due to incomplete absorption of 2,3,7,8-TCDD after oral exposure. Although these results need further substantiation, it is conceivable that the sex of the animal, pregnancy and/or the route of exposure could have a significant impact on the pharmacokinetics of these compounds.

In a related study, Krowke (1986) compared the 2,3,7,8-TCDD concentrations in the liver of pregnant and nonpregnant NMRI mice exposed subcutaneously to 12.5 or 25 nmol/kg/day on gestation days 9-11. At 7 days after exposure to the lower dose, the hepatic 2,3,7,8-TCDD concentrations were 7 and 32 ng/g in pregnant and nonpregnant mice, respectively. At the higher exposure, 5.5 times lower concentrations of 2,3,7,8-TCDD were found in the livers of pregnant animals on gestation day 18. A similar effect on hepatic 2,3,7,8-TCDD levels was observed also in combined exposure, which contained 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD or 2,3,7,8-TCDF. The decreased hepatic levels of 2,3,7,8-TCDD in pregnant mice are consistent with the Weber and Birnbaum (1985) observation of more rapid elimination of 2,3,7,8-TCDD in pregnant mice. Further investigations are necessary to better characterize the apparently significant effects of pregnancy on the disposition of 2,3,7,8-TCDD and related compounds.

Weber and Birnbaum (1985) also investigated the distribution of [^{14}C]-2,3,7,8-TCDD (30 μg/kg) and [^{14}C]-2,3,7,8-TCDF (800 μg/kg) to the embryos of pregnant C57BL/6N mice after oral exposure on gestation day 11. On gestation days 12, 13 and 14, the percent of the maternal dose in the embryo remained constant at 0.032-0.037%/ embryo, while the concentrations in the embryo were 0.34, 0.17 and 0.15% of the dose/g embryo, respectively. Embryos had approximately 11-fold higher concentrations of 2,3,7,8-TCDD than 2,3,7,8-TCDF when exposed on a percent of total dose/g tissue basis. This may be due to the more rapid metabolism and excretion of 2,3,7,8-TCDF compared to 2,3,7,8-TCDD. Assuming that all radioactive material found in embryos was parent compound, at most, 2.6 ng (8 pmol) of 2,3,7,8-TCDD and 6.4 ng (21 pmol) of 2,3,7,8-TCDF/g tissue were detected under these conditions.

The transfer of [^{14}C]-2,3,7,8-TCDD to the embryo during early gestation was assessed in NMRI mice given a dose of 25 μg/kg by intraperitoneal injection on either days 7, 8, 9, 10, 11 or 13 of gestation (Nau and Bass, 1981). The mice were sacrificed after 48 hours, and 2,3,7,8-TCDD concentrations were determined by liquid scintillation counting of solubilized tissue and by GC-ECD and GC/MS. Similar results were given by these methods, suggesting that 2,3,7,8-TCDD derived [^{14}C] in maternal and embryonic tissue was the parent compound. The maternal liver contained from 4-8% of the dose/g or 40-80 ng/g. 2,3,7,8-TCDD in embryonic tissue from gestation days 11-15 ranged from 0.04-0.1% of the dose/g or 0.4-1.0 ng/g. In contrast, higher levels were found earlier in gestation, with 10 ng/g embryo on gestation day 9 and 2 ng/g on day 10. The higher levels may be related to placentation, which occurs at approximately gestation days 10-11 in this mouse strain. The affinity of fetal liver for 2,3,7,8-TCDD was relatively low, as compared to maternal liver; however, 2,3,7,8-TCDD levels in fetal livers were 2-4 times higher than levels in other fetal organs. Nau and Bass (1981) also attempted to correlate 2,3,7,8-TCDD levels in the fetuses with the observed incidence of cleft palate. Three groups of mice were given either a single intraperitoneal exposure to 25 μg/kg 2,3,7,8-TCDD on gestation day 7 or 10 or 5 μg/kg/day, intraperitoneally, on gestation days 7-11. On gestation day 13, 2,3,7,8-TCDD concentrations in maternal tissues were very similar in the three

exposure groups. At day 13, however, the embryo contained 0.038±0.011% (0.36 ng/g), 0.096±0.027% (0.92 ng/g) and 0.12±0.05% (1.1 ng/g) of the dose (mean±SD) in the 7-, 10- and 7- to 11-day exposure groups, respectively. Cleft palate incidence on gestation day 18 was 16, 84 and 65% for the 7-, 10- and 7- to 11-day exposure groups, respectively. Although further studies are needed, these results suggest that cleft palate incidence is generally related to the 2,3,7,8-TCDD concentration in the embryo. In a related study, Couture et al. (1990) found that gestation day 12 was the peak period of sensitivity for 2,3,7,8-TCDD-induced cleft palate in C57BL/6N mice; however, tissue levels were not investigated.

In the same laboratory, Abbott et al. (1989) investigated the distribution of 2,3,7,8-TCDD in the C57BL/6N mouse fetus following maternal exposure on gestation day 11 to 30 μg/kg. 2,3,7,7-TCDD was detected in the gestation day-11 embryo at 3 hours post-exposure and was equally distributed between the embryonic head and body. At 72 hours post-exposure, 0.035% of the total dose was in fetal tissues, and 1% of the 2,3,7,8-TCDD in the fetus (1.4-3.5 pg was found in the palatal shelf.

Krowke (1986) also measured the concentration of 2,3,7,8-TCDD in the placenta, amniotic fluid and fetus of NMRI mice exposed to 2.5 nmol/kg by subcutaneous injection on days 9-11 of gestation. Similar concentrations of 2,3,7,8-TCDD were observed in the placenta, amniotic fluid and fetus (~0.5 ng/g) on day 16 of gestation. Fetal liver 2,3,7,8-TCDD concentrations were at least five times greater than other fetal tissue. Krowke (1986) reported slightly lower 2,3,7,8-TCDD levels in the fetal head relative to other extrahepatic fetal tissue, while Weber and Birnbaum (1985) found a slightly higher 2,3,7,8-TCDD concentration in the head relative to other extrahepatic fetal tissue.

Nau et al. (1986) investigated the transfer of 2,3,7,8-TCDD via the placenta and milk in NMRI mice exposed to 25 μg/kg on day 16 of gestation. The authors confirmed the relatively low fetal tissue levels with prenatal exposure to 2,3,7,8-TCDD (Nau and Bass, 1981) and found that postnatally, 2,3,7,8-TCDD was transferred efficiently to mouse neonates and offspring by lactating mothers. During the first 2 postnatal weeks, the pups were given doses of 2,3,7,8-TCDD via the milk that were, on a body weight basis, similar to those that had been

administered prenatally to their mothers. 2,3,7,8-TCDD levels in the tissue of lactating mothers decreased within the first 3 postnatal weeks by two to three orders of magnitude to reach levels that were only ~2% of the corresponding levels in the pups that these mothers had nursed. Thus in mice, excretion into milk represents a major pathway for maternal elimination of 2,3,7,8-TCDD and for the subsequent exposure of pups.

The disposition of 2,3,7,8-TCDD in rat pups was assessed after the prenatal (via placental transfer) and/or postnatal (via milk) exposure from pregnant Wistar rats given a single dose of 3, 30 or 300 ng/kg, subcutaneously, on day 19 of gestation (Korte et al., 1990). Lactation resulted in the rapid elimination of 2,3,7,8-TCDD from maternal tissues, with the half-life of 2,3,7,8-TCDD in the liver of lactating rats estimated to be ~7 days. This compares to a half-life of 13.6 days in the liver of nonlactating rats (Abraham et al., 1988). At postnatal day 7, exposure via the milk resulted in pup liver 2,3,7,8-TCDD concentrations that were greater than the corresponding levels in maternal liver. In cross-fostering experiments, the concentrations of 2,3,7,8-TCDD in the liver of offspring at postnatal day 7 were 0.47, 2.59 and 4.16 ng/g in the 300 ng/kg groups exposed through the placenta only, via the milk only and through the placenta and via the milk, respectively. These results support the earlier observations that the placental transfer of 2,3,7,8-TCDD in rats and mice is relatively limited compared with the efficient transfer via maternal milk.

Van den Berg et al. (1987) investigated the transfer of CDDs and CDFs to fetal and neonatal rats. Prenatal exposure of the fetus was assessed in pregnant Wistar rats fed a diet containing a fly ash extract from a municipal incinerator on days 10-17 of gestation. Postnatal exposure of 10-day-old pups was assessed through feeding lactating mothers the same contaminated diet for the first 10 days after delivery. Although the fly ash extract contained almost all of the 136 tetra- to octa-CDDs and -CDFs, only 17 CDD and CDF congeners were detected as major compounds in the tissue of fetuses, pups and dams. All of the congeners were 2,3,7,8-substituted with the exception of 2,3,4,6,7-PeCDF. 2,3,7,8-TCDD had the highest retention (0.0092% of the dose/g) in the fetus, while 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF and hepta- and octa-CDDs and -CDFs were not detected in the fetus. In the liver of offspring, the highest retention was found for 2,3,7,8-

TCDD, 1,2,3,7,8-PeCDD and the three 2,3,7,8-substituted HxCDDs (0.74-1.13% dose/g). The 2,3,7,8-substituted penta- and hexachlorinated congeners showed the highest retention in the livers of dams (2.05-5.17% of dose/g liver), while 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF and 2,3,4,6,7-PeCDF had the lowest retention. A linear relationship was found between the retention of CDDs and CDFs in the livers of pregnant and lactating rats. Furthermore, a linear relationship was found between the retention of CDDs and CDFs in the livers of the lactating rats and livers of the offspring.

In a related study, Hagenmaier et al. (1990) investigated the transfer of CDDs and CDFs through the placenta and via milk in a marmoset monkey. A defined mixture of CDDs and CDFs was given as a single subcutaneous injection to a pregnant marmoset monkey at the end of the organogenesis period (week 10 of gestation, 11 weeks prior to delivery). Transfer of CDDs and CDFs through the placenta was investigated in a newborn 1 day after birth, and transfer through the placenta and via milk was assessed in an infant of the same litter after a lactation period of 33 days. Tissue concentrations of the offspring were compared with those of the mother at the end of the lactation period and with data from other adult marmosets obtained at this time of maximum absorption (1 week after injection) and 6 weeks after injection. Deposition of CDDs and CDFs into the newborn liver was very low, suggesting very little transplacental transport and hepatic accumulation of these compounds. 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD were found at the highest concentration in the liver of the newborn (~0.15% of dose/g). For all other congeners, the concentrations in the liver of the newborn were <10% of the corresponding concentrations in adults. In contrast to liver, concentrations of 2,3,7,8-substituted congeners in the adipose tissue of the newborn were at least 33% of the levels in adults, and in the case of OCDD and OCDF, levels were 3-fold higher in the newborn than in the adult. The adipose tissue/liver concentration ratios for 2,3,7,8-substituted congeners in the newborn ranged from 2.2 for 1,2,3,4,6,7,8-HpCDF to 10.9 for 2,3,7,8-TCDF. Furthermore, the concentration of these congeners in the newborn was highest in the adipose tissue, followed by the skin and liver. This is in contrast to the relative distribution in the adult where the liver generally contains the highest levels of these congeners. The results indicate that

hepatic concentrations in the fetus may not be representative of the rate of placental transfer of CDDs and CDFs. In the marmoset monkey, substantial placental transfer into fetal adipose tissue can be observed for most of the 2,3,7,8-substituted congeners during the fetal period. As expected from rodent studies, the transfer of CDDs and CDFs via mothers' milk was considerable, resulting in hepatic concentrations of 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 1,2,3,6,7,8-HxCDD in the suckled infant (postnatal day 33) higher than those in the dam. The hepatic concentration of 2,3,7,8-TCDD in the 33-day-old infant was ~0.9% of the dose/g tissue. Transfer of hepta- and octa-CDDs and CDFs to the suckled infant was rather low, only ~10% of the levels in the dam. When total exposure of the mother and offspring at the end of the 33-day nursing period was assessed in terms of I-TE factors (U.S. EPA, 1989), the liver of the mother contained 2494 pg I-TE/g, while the offspring liver contained 2022 pg I-TE/g. This approach is necessary to assess total exposure due to the congener-specific transfer via lactation.

The pre- and postnatal transfer of 2,3,7,8-TCDD to the offspring of rhesus monkeys was investigated by Bowman et al. (1989). Animals were fed a diet containing 2,3,7,8-TCDD at concentrations of 5 or 25 ppt for ~4 years and were on a 2,3,7,8-TCDD-free diet for ~18 months prior to parturition. Maternal 2,3,7,8-TCDD levels (mean±SE) in adipose tissue were 49±11 (n=7) and 173±81 (n=3) ppt in the 5 and 25 ppt groups, respectively. Corresponding levels in the adipose tissue of offspring at weaning (4 months) were 187±58 an 847±298 ppt in the 5 and 25 ppt groups, respectively. From these data, a 2,3,7,8-TCDD BCF of 4.29 was estimated from mother to nursing infant. This value is similar to that observed for 2,3,7,8-TCDD in the marmoset monkey (Hagenmaier et al., 1990). The milk of the rhesus monkeys in the 25 ppt group contained from 4-14 ppt of 2,3,7,8-TCDD, which corresponds to 150-500 ppt on a lipid basis. The authors calculated that the three mothers in the 25 ppt group excreted from 17-44% of their 2,3,7,8-TCDD body burden by lactation. They also concluded that the results are generally consistent with overall triglyceride movement as mediating the excretion of 2,3,7,8-TCDD in milk.

In a subsequent study, Bowman et al. (1990) reported the relative persistence of 2,3,7,8-TCDD in the offspring of rhesus monkeys that were exposed

earlier to 5 or 25 ppt of 2,3,7,8-TCDD in the diet. The concentration of 2,3,7,8-TCDD in adipose tissue was measured in offspring at ~4-5, 12 and 24 months of age. The decrease of 2,3,7,8-TCDD levels in adipose tissue of seven young monkeys departed somewhat from first-order, single-compartment kinetics, but with the limited data and an assumption of first-order kinetics, a half-life of 121 days was estimated. When the data were adjusted within each animal for body weight gain and for average fat content at each age, the adjusted data apparently followed first-order, single-compartment kinetics, with a half-life of ~181 days. Thus, young monkeys apparently eliminate 2,3,7,8-TCDD from adipose tissue at a faster rate than adult rhesus monkeys, which had individual half-lives ranging from 180-550 days (Bowman et al., 1989).

Furst et al. (1989) examined the levels of CDDs and CDFs in human milk and the dependence of these levels on the period of lactation. The mean concentrations of CDDs in human milk (on a fat basis) ranged from 195 ppt for OCDD to 2.9 ppt for 2,3,7,8-TCDD, with the levels of the other congeners decreasing with decreasing chlorination. This is in contrast to the generally lower levels of CDFs in human milk, which range from 25.1 ppt for 2,3,4,7,8-PeCDF to 0.7 ppt for 1,2,3,7,8-PeCDF. An evaluation of the CDD and CDF levels in relation to the number of breast-fed children found that the concentrations in milk generally decreased with the greater number of children. The CDD and CDF levels in milk from mothers nursing their second child are on average 20-30% lower than those for mothers breast-feeding their first child. CDD and CDF levels were also analyzed in one mother over a period of 1 year after delivery of her second baby to assess the effect of duration of lactation. After breast-feeding for 1 year, the mother had CDD and CDF levels that were 30-50% of the starting concentration. Levels in milk fat (ppt) at 1, 5 and 52 weeks after delivery were 251, 132 and 119 for OCDD, 7.9, 5.9 and 1.4 for 2,3,7,8-TCDD and 33.1, 24.5 and 10 for 2,3,4,7,8-PeCDF, respectively. The results suggest a more rapid mobilization of CDDs and CDFs and excretion into human milk during the first few weeks post-partum. Although further studies are necessary, the limited data suggest that there are time-dependent, isomer-specific differences in the excretion of CDDs and CDFs in human milk.

Although data are more limited for the coplanar PCBs, 3,3',4,4'-TCB, 3,3',4,4',5-PeCB and 3,3',4,4',5,5'-HxCB have been detected in human milk from Swedish mothers, at concentrations of 16-32, 72-184 and 46-129 ppt on a fat basis, respectively (Noren et al., 1990). Therefore, lactation appears to be an effective means for the excretion of coplanar PCBs from mothers and a major source of postnatal exposure of nursing infants. Since 3,3',4,4',5-PeCB and other coplanar PCBs are present in human milk at concentrations up to 60-fold higher than 2,3,7,8-TCDD, it is important to consider the relative toxic potency of these dioxin-like compounds and their potential health impact on nursing infants.

1.5.2. Aging. The influence of aging on the intestinal absorption of 2,3,7,8-TCDD was studied in 13-week-, 13-month- and 26-month-old (senescent) male Fischer 344 rats (Hebert and Birnbaum, 1987). Absorption was measured by an *in situ* intestinal recirculation perfusion procedure. When absorption was calculated in terms of ng 2,3,7,8-TCDD absorbed/g mucosal dry weight/hour, the decrease between the senescent rats and the two younger age groups, from 544 ng/g/hour (young) to 351 ng/g/hour (senescent), was not statistically significant ($p<0.05$). The results indicate that, as with other molecules that depend on diffusion for their absorption, aging does not affect the intestinal absorption of 2,3,7,8-TCDD.

Banks et al. (1990) studied the effect of age on the dermal absorption and disposition of 2,3,7,8-TCDD and 2,3,4,7,8-PeCDF in male Fischer 344 rats. When rats were administered the same dose per body weight, dermal absorption of 2,3,7,8-TCDD, at 3 days after exposure, decreased from 17.7±2.7% (mean±SD) to 5.6±2.5% of the administered dose in 10- and 36-week-old rats, respectively. Dermal absorption in the 96-week-old rats was similar to that of the 36-week-old rats. Dermal absorption of 2,3,4,7,8-PeCDF also decreased from 22.2±0.2 to 14.7±3.8% of the administered dose in 10- and 36-week-old rats, respectively. Dermal absorption of both compounds was also decreased in older rats given the same total dose per surface area. Older animals may have decreased blood flow in the upper dermis, which will decrease the clearance of these compounds from the application site. Potential age-related changes in the intercellular stratum corneum lipids may also play a role in the decreased dermal absorption observed in older animals. Changes in the percentage of the administered dose detected

in various depots reflected age-related changes in dermal absorption, while age-related changes in the tissue distribution of the absorbed dose reflected changes in the total mass of these tissues at various ages. Overall elimination of the absorbed dose was not affected by age. Although this investigation was conducted using a lipophilic solvent system and an animal model with skin that is more permeable than human skin, the results suggest that systemic bioavailability after dermal exposure to 2,3,7,8-TCDD or 2,3,4,7,8-PeCDF may be reduced in older age groups.

In a similar study, absorption, tissue distribution and elimination were examined 72 hours after dermal application of a lower dose of 200 pmol (111 pmol/cm^2) 2,3,7,8-TCDD to weanling (3-week-old), juvenile (5-week-old), pubescent (8-week-old), young adult (10-week-old) and middle-aged (36-week-old) rats (Anderson et al., 1992). Dermal absorption using acetone as vehicle was greatest in 3-week-old rats (129 pmol; 64% of the administered dose), decreasing to ~80 pmol (40%) in 5-, 8- and 10-week-old rats and to 45 pmol (22%) in 36-week-old rats. The results indicate that 2,3,7,8-TCDD is absorbed to a greater degree through skin of very young animals and that a significant decrease in potential for systemic exposure may occur during maturation and again during aging.

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