

Domestic Policy - Brian Burke

Box 3

1. dioxin materials
2. Business Council on Indoor Air
3. Climate Change Action Plan/Climate Change mitigation group
4. Yucca Mountain Background material and Presidential press clippings
5. Car Talk Material

ENCLOSURES FILED OVERSIZE ATTACHMENTS

4432

NARA 3092

Down

PHOTOCOPY
PRESERVATION



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

May 20, 1994

OFFICE OF
RESEARCH AND DEVELOPMENT

TO: Brian Burke 6/15/94

Interagency Reviewers of the EPA Dioxin Risk Characterization Chapter

I sent you a copy of the dioxin risk characterization chapter about two weeks ago and requested your review and comments on the chapter. Since that time, we have been asked to provide you and all of the other interagency reviewers with a copy of the complete set of the draft chapters that will comprise the Dioxin Health Assessment Document plus the draft of the companion Dioxin Exposure Assessment Document. These draft documents are enclosed with this note.

These documents are provided to you for two purposes: (1) to provide additional background and reference materials to support your review of the risk characterization chapter, and (2), to enable you to track how comments that you or your Agency may have submitted previously were dealt with in the current version of the document. We consider the Exposure Assessment Document and most of the chapters of the Health Assessment Document to be ready for printing as external review drafts. The main focus of your review should continue to be the risk characterization chapter; we would prefer to hold off on a detailed review of the other health chapters and the Exposure Assessment Document and accept recommendations for any non-substantive changes during the External Review period. You should have separately received information on due dates and submission procedures for your review.

Please note that at this stage all of the draft Health Assessment Document chapters and the three volume Exposure Assessment Document should be regarded as drafts in progress, and as such should not be quoted, cited, or released in any manner. No additional copies should be made and the information and conclusions contained in the draft should not be shared with others.

As I explained in my earlier transmittal, once we have gotten comments from the interagency reviewers and the revisions for these comments have been incorporated, the revised Risk Characterization chapter will be printed and issued as Chapter 9 in the External Review Draft of the full Health Assessment Document. The External Review Draft of the Health Assessment Document, along with the External Review Draft for the Dioxin Exposure Assessment Document, will be released for a 90-day public review and comment period. The dates and availability of documents will be announced in the *Federal Register*.

Thank you for your participation in this review. If you have any questions, please feel free to contact me by phone at 202-260-7315 or by fax at 202-260-0393.

Sincerely,

A handwritten signature in dark ink, appearing to read "William H. Farland".

William H. Farland, Ph.D.
Director, Office of Health and
Environmental Assessment (8601)

DRAFT--DO NOT QUOTE OR CITE

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

TABLE OF CONTENTS

Volume I

1. DISPOSITION AND PHARMACOKINETICS
2. MECHANISMS OF ACTION
3. ACUTE, SUBCHRONIC, AND CHRONIC TOXICITY
4. IMMUNOTOXICITY OF TCDD AND RELATED HALOGENATED AROMATIC HYDROCARBONS
5. DEVELOPMENTAL AND REPRODUCTIVE TOXICITY
6. CARCINOGENICITY OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) IN ANIMALS

Volume II

7. EPIDEMIOLOGY/HUMAN DATA
 - PART I. CANCER EFFECTS
 - PART II. HEALTH EFFECTS OTHER THAN CANCER
8. DOSE-RESPONSE MODELING FOR 2,3,7,8-TCDD

Volume III

9. RISK CHARACTERIZATION OF DIOXIN AND RELATED COMPOUNDS

Chapter 1. Disposition and Pharmacokinetics

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

NOTICE

THIS DOCUMENT IS A PRELIMINARY DRAFT. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

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

DRAFT--DO NOT QUOTE OR CITE

DISCLAIMER

This document is an internal draft for review purposes only and does not constitute Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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. Several reviews on this subject focus on 2,3,7,8-TCDD and related halogenated aromatic hydrocarbons (Neal et al., 1982; Gasiewicz et al., 1983b; Olson et al., 1983; Birnbaum, 1985; Van den Berg et al., 1994). During the past 6 years, considerably more data have been published on this class of compounds, which includes 2,3,7,8-substituted CDDs, BDDs, CDFs, BDFs, and the coplanar 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

Gastrointestinal, dermal, and transpulmonary absorptions represent potential routes for human exposure to this class of persistent environmental contaminants. Parenteral absorption is a route of exposure that 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 percent of the dose that was not absorbed. The unabsorbed fraction is estimated as the recovery of parent compound in feces within 24 to 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.

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		Vehicle	% Administered Dose Absorbed ^a [Mean (Range)]	Reference
		(μmol/kg)	(μg/kg)			
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	50	o-dichlorobenzene:Emulphor (1:1)	12	Birnbaum and Couture, 1988
		1.1	500		15	
		1.1	500	o-dichlorobenzene:corn oil (1:1)	2	
		11	5000	oil (1:1)	5	
				corn oil suspension		
				corn oil suspension		

TABLE 1-1 (cont.)						
Chemical	Species (Sex)	Dose		Vehicle	% Administered Dose Absorbed* [Mean (Range)]	Reference
		(μmol/kg)	(μg/kg)			
BDDs						
2,3,7,8-TBDD	Fischer 344 rat (M)	0.001	0.5	Emulphor:ethanol:water (1:1:3)	78	Diliberto et al., 1990
		0.01	5		82	
		0.1	50		60	
		0.5	500		47	
CDFs						
2,3,7,8-TCDF	Fischer 344 rat (M)	0.1	30.6	Emulphor:ethanol (1:1)	90	Birnbaum et al., 1980
		1.0	306		90	
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	34	corn oil	~ 70	Brewster and Birnbaum, 1987
		0.5	170		~ 70	
		1.0	340		~ 70	
PCBs						
3,3'4,4'-T4CB	C57BL mouse (F)	34.5	10,000	corn oil	77	Wehler et al., 1989

*Absorption is generally estimated as the difference between the administered dose (100%) and the percent 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.

In Sprague-Dawley rats given a single oral dose of $1.0 \mu\text{g}$ [^{14}C]-2,3,7,8-TCDD/kg bw in acetone:corn oil (1:25, v/v), the fraction absorbed ranged from 66% to 93%, with a mean of $\sim 84\%$ (Rose et al., 1976). With repeated oral dosing of rats at 0.1 or $1.0 \mu\text{g/kg/day}$ (5 days/week for 7 weeks), gastrointestinal absorption 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 ($50 \mu\text{g/kg}$) resulted in an average absorption of 70% of the administered dose (Piper et al., 1973).

One study in the guinea pig reported that $\sim 50\%$ 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., 1980). Hamsters were given a single, sublethal, oral dose of [$1,6\text{-}^3\text{H}$]-2,3,7,8-TCDD in olive oil ($650 \mu\text{g/kg}$), 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 $1.4 \mu\text{g/kg/day}$) for 42 days, 50% to 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 [^3H]-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 before 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 $5 \mu\text{g/kg}$ 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 days 1 and 2 — % total intravenous dose in feces on days 1 and 2) using the intravenous pharmacokinetic data of Kedderis et al. (1991a).

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 $0.5 \mu\text{mol/kg}$.

respectively. These results suggest nonlinear absorption at the higher doses, with maximal oral absorption at an exposure of $\leq 0.01 \mu\text{mol/kg}$ ($5 \mu\text{g/kg}$).

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 $1.0 \mu\text{mol/kg}$) 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 ($0.2 \mu\text{mol/kg}$, $6 \mu\text{g/kg}$, and $1\text{--}15 \mu\text{g/kg}$) 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 with 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 was 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 $1.0 \mu\text{mol/kg}$). Incomplete and variable absorption of 1,2,3,7,8-PeCDD was reported in rats, with 19% to 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% to 15% of the administered dose. Lower doses ($50 \mu\text{g/kg}$) in an 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 $105 \text{ ng } [^3\text{H}]\text{-}2,3,7,8\text{-TCDD}$ (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 2,120 days.

The above data indicate that gastrointestinal absorption of 2,3,7,8-TCDD and related compounds is variable, incomplete, and congener and vehicle specific. More soluble congeners, such as 2,3,7,8-TCDF, are almost completely absorbed, while the extremely insoluble OCDD is 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 database also suggests that there are no major interspecies differences in the gastrointestinal absorption of these compounds.

Because CDDs, CDFs, and PCBs are present in human milk, McLachlan (1993) investigated the net absorption of these compounds in a nursing infant. The contaminant input, through the ingestion of mother's milk, and the contaminant output in the feces were measured to estimate the digestive tract absorption of these compounds. For almost all congeners, more than 90% of the ingested compound was absorbed, indicating that the common assumption of 100% absorption of CDDs, CFFs, and PCBs in nursing infants is reasonable.

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

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 $\pm$ 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 $\pm$ 4.8
8 days	21.2, 22.7	10	16.0 $\pm$ 2.2
Aqueous suspension of activated carbon (25%, w/w)	14.7	6	$\leq$ 0.07

*Source: Poiger and Schlatter, 1980.
w/w = Weight by weight.

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 Huetter 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., 1987a). 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 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.

Because 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 with that from 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 soil from Times Beach and Minker Stout, Missouri, was $\sim 50\%$ less than that from corn oil. Shu et al. (1988a) reported an oral bioavailability of $\sim 43\%$ 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 2-1,450 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 in 60 μL of acetone was then applied over a 1.8 cm^2 area of skin, which then was 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 $\mu\text{mol/kg}$, 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 ($\mu\text{g/kg}$) 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 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} . With 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 ($\leq 0.1 \mu\text{mol/kg}$), a greater percentage 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,

TABLE 1-3				
Dermal Absorption of 2,3,7,8-TCDD and Related Compounds in the Rat ^a				
Chemical	Dose		% Administered Dose	
	(μ mol/kg)	(μ g/kg)	Skin Site ^b	Absorbed
2,3,7,8-TCDD	0.00015	0.05	61.73 $\pm$ 4.37	38.27 $\pm$ 4.37
	0.001	0.32	59.71 $\pm$ 1.90	40.29 $\pm$ 1.89
	0.01	3.2	72.60 $\pm$ 0.41	27.40 $\pm$ 0.41
	0.1	32	82.21 $\pm$ 2.85	17.78 $\pm$ 2.85
	0.5	160	80.92 $\pm$ 2.74	19.08 $\pm$ 2.74
	1.0	321	82.68 $\pm$ 3.69	17.30 $\pm$ 3.67
2,3,7,8-TCDF	0.1	31	51.18 $\pm$ 11.95	48.84 $\pm$ 11.95
	0.5	153	82.14 $\pm$ 11.22	17.86 $\pm$ 11.22
	1.0	306	88.70 $\pm$ 5.17	11.32 $\pm$ 5.17
1,2,3,7,8-PeCDF	0.1	34	74.72 $\pm$ 3.58	25.27 $\pm$ 3.58
	0.5	170	91.67 $\pm$ 2.46	8.33 $\pm$ 2.46
	1.0	340	84.23 $\pm$ 5.44	15.76 $\pm$ 5.44
2,3,4,7,8-PeCDF	0.1	34	65.77 $\pm$ 4.80	34.19 $\pm$ 4.78
	0.5	170	75.50 $\pm$ 1.81	24.50 $\pm$ 1.80
	1.0	340	81.84 $\pm$ 1.67	18.16 $\pm$ 1.67

^aSource: Brewster et al., 1989.

^bValues are the mean $\pm$ 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.

occurs at a very slow rate. Using a similar exposure protocol, the dermal absorption of 2,3,7,8-TBDD was only 30% to 40% of that observed for 2,3,7,8-TCDD (Diliberto et al., 1993a).

The dermal absorption of several polyhalogenated aromatic hydrocarbons in male F344 rats was also compared with estimates of their respective octanol-water partition coefficients (Jackson et al., 1993). Inverse correlations were found between octanol-water partition coefficient estimates and the single dose (~ 0.1 $\mu\text{g}/\text{kg}$) dermal absorption for most of the compounds studied. The differential dermal absorption of 2,3,7,8-TCDD and 2,3,7,8-TBDD may result, in part, from the diminished ability of the more lipophilic 2,3,7,8-TBDD to partition out of the stratum corneum and into the underlying epidermal and dermal layers.

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 percentage 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 because the viable tissues were major barriers 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 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 because 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 to 170 pg/hour/cm², while penetration into the dermis and epidermis ranged from 100 to 800 pg/hour/cm². 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.

Wester et al. (1993a) studied the dermal permeation potential of two PCBs, Aroclor 1242 and Aroclor 1254, from soil. Soil is the most common medium of contact for humans; hence, any permeation potential is of great interest for risk assessment purposes. This study consisted of experiments conducted in rhesus monkeys (in vivo), in vitro human skin, and powdered human corneum. The monkeys were exposed topically for a 5-week period. Percutaneous absorption was determined by urinary and fecal [¹⁴C]-PCB excretion. The percutaneous absorption in the monkey was 13.8% ($\pm 2.7\%$) for Aroclor 1242 and 14.1% ($\pm 1.0\%$) for Aroclor 1254. The authors report that these absorption rates for soil are similar to those from vehicles such mineral oil, trichlorobenzene, and acetone. These rates are somewhat higher than those for other compounds such as the pesticide butachlor, which was about 5.0% when applied to human skin (Ademola et al., 1993). On the other hand, Wester et al. (1993b) report a much higher absorption for pentachlorophenol (PCP) (24.4% from soil and 29.2% from acetone) in the rhesus monkey. The authors report that PCP is one of the more extensively absorbed compounds they have studied. Thus, at least for these two PCBs (Aroclor 1242 and Aroclor 1254), the absorption in the rhesus monkey appears to be intermediate between that of other compounds tested. In summary, these studies indicate that these two PCBs show significant partitioning into the stratum corneum from soil, a common environmental matrix.

1.1.2.1. *Bioavailability Following Dermal Exposure*

Dermal exposure of humans 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

DRAFT--DO NOT QUOTE OR CITE

compared with absorption of pure compound dissolved in solvents. In a control experiment, 26 ng of 2,3,7,8-TCDD in 50 μ L methanol was administered to the skin of rats; 24 hours later the liver contained $14.8 \pm 2.6\%$ 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 $\sim 40\%$ 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 that are based solely on hepatic levels following exposure to 2,3,7,8-TCDD. As compared with dermal application in methanol, dermal application to rats of 2,3,7,8-TCDD 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 ($\sim 1 \mu\text{g}/\text{ear}$) 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 $\sim 2\%$ 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 $\sim 160 \mu\text{g}$. These results suggest that dermal absorption of 2,3,7,8-TCDD depends on the formulation (vehicle or adsorbent) containing the toxin.

Shu et al. (1988b) 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 $\sim 60\%$ 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, by the presence of crankcase oil as co-contaminants, or by environmentally versus laboratory-contaminated soil.

A major limitation of these 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 indicated 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 on dermal uptake (Gallo et al., 1992). Because 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, data on the rhesus monkey are very limited. 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 in female Sprague-Dawley rats after intratracheal instillation of the compound 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 $\geq 0.55 \mu\text{g TCDD/kg}$. 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 pulmonary bioavailability of 2,3,7,8-TCDD was also examined in female Sprague-Dawley rats following intratracheal instillation of PCDD-contaminated soil from a former 2,4,5-trichlorophenoxy-acetic acid manufacturing site (Nessel et al., 1992). A size-dependent enrichment of PCDDs and PCDFs was observed, with the smaller particles being more highly contaminated. 2,3,7,8-TCDD was enriched up to 33-fold as compared with unfractionated soil in small respirable particles. Pulmonary bioavailability of 2,3,7,8-TCDD was assessed by hepatic enzyme inductions (AHH activity) and 2,3,7,8-TCDD concentration. The data indicate that the relative pulmonary bioavailability of 2,3,7,8-TCDD on respirable soil particles is 100% as compared with laboratory recontaminated gallium oxide.

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 $\sim 92\%$, 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., 1993a,b). 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. (1989a,b) used various vehicles to investigate the absorption of 2,3,7,8-TCDD after parenteral application in rats. These investigators observed optimal results with the subcutaneous injection of 2,3,7,8-TCDD with a mixture of toluene:DMSO (1:2) as the 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 with toluene:DMSO (1:2) as a vehicle. Of the 97 congeners analyzed, 70 were $\geq 95\%$ 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., 1989a). 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., 1989a). 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. Brunner et al. (1989) also reported that intraperitoneal administration of CDDs and CDFs revealed a delayed absorption from the abdominal cavity that 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. This may also be true following oral exposure due to different perfusion rates of different fat depots (McKinley et al., 1993).

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 after 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. A subsequent in vivo investigation reported a similar distribution of 2,3,7,8-TCDD in the various fractions of human whole blood (Patterson et al., 1989b). 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 [^3H]-2,3,7,8-TCDD among lipoprotein fractions from three fasting, normolipemic donors indicated a greater percentage associated with LDL

($55.3 \pm 9.03\%$ SD) than with VLDL ($17.4 \pm 9.07\%$ SD) or HDL ($27.3 \pm 10.08\%$ 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).

Congener-specific differences have been observed for the in vivo binding of the 2,3,7,8-substituted PCDDs and PCDFs to different serum fractions in human blood (Patterson et al., 1989b). Binding to the lipoproteins gradually decreased with increasing chlorine content, with about 75% of 2,3,7,8-TCDD bound to lipoproteins, while approximately 45% of OCDD was bound to this fraction. In contrast, binding to other serum proteins increased with chlorine content from approximately 20% for 2,3,7,8-TCDD to 50% for OCDD. The results indicate that the higher chlorinated PCDDs and PCDFs do not partition according to the lipid content of these blood fractions.

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 that in normolipidemic animals, and this may contribute to the plasma lipoprotein binding modifying the toxicity of 2,3,7,8-TCDD in hyperlipidemic rats.

The time- and temperature-dependent cellular uptake of lipoprotein-associated 2,3,7,8-TCDD by cultured human fibroblasts 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, as 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 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 gram tissue and as percent of dose per organ. Liver, adipose tissue, skin, and thyroid were the only tissues 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., 1983a; 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

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

^aSource: Abraham et al., 1988.

^bDistribution was assessed 7 days after a single subcutaneous exposure (3 µg/kg bw).

distribution of related compounds. 2,3,7,8-TBDD and 1,2,3,7,8-PeCDD disposition in the rat was very similar to that of 2,3,7,8-TCDD (Kedderis et al., 1991a; 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 μ g 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 these congeners than were detected in kidney, brain, heart, and testes. Unexpectedly, the concentrations of these 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 in 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 mRNA 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 localization 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., 1978a); 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 because 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 because 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 [³H]-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, Missouri, by Graham et al. (1986); in Atlanta, Georgia, 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 high-resolution gas chromatographic/high-resolution mass spectrometric analysis for 2,3,7,8-TCDD in human serum. The arithmetic mean of the individual human serum samples was 47.9 ppt 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), Kahn et al. (1988), and Schecter et al. (1990b). All three 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.

Congener-specific partitioning of 2,3,7,8-substituted PCDDs and PCDFs between adipose tissue and plasma lipids has also been reported in a study of 20 Massachusetts Vietnam veterans (Schecter et al., 1990b). The distribution ratio between plasma lipid and adipose tissue increased with chlorine substitution on the PCDDs and PCDFs. While 2,3,7,8-substituted TCDD, TCDF, PeCDD, PeCDF, HxCDD, and HxCDF had a plasma lipid to adipose tissue ratio of about 1.0, OCDD had a ratio of 2.0. On the other hand, whole blood PCDDs and PCDFs seem to be found at the same concentrations as in adipose tissue on a lipid basis (Schecter, 1991).

The disposition of 2,3,7,8-substituted PCDDs and PCDFs in human liver and adipose tissue was assessed in a study of 28 people from the Munich area (Thoma et al., 1989; 1990). Table 1-X summarizes these results, which are expressed both on a lipid and wet weight basis. The concentrations of PCDDs and PCDFs in adipose tissue and liver are not the same when calculated on a lipid basis. This is in contrast to the high correlation that was reported between adipose tissue and serum TCDD levels when expressed on a lipid-weight basis (Patterson et al., 1988; Kahn et al., 1988; Schecter et al., 1990b). Furthermore, the liver/adipose tissue ratio increased with the higher chlorinated PCDDs and PCDFs. The congener-specific hepatic disposition is also similar to that observed in rats and marmoset monkeys exposed to a complex mixture of PCDDs and PCDFs (Abraham et al., 1989b; Neubert et al., 1990). Therefore, it is important to consider congener- and tissue-specific differences in disposition of PCDDs and PCDFs when blood levels are used to estimate tissue levels or body burdens.

In a study of potentially heavily exposed Vietnam veterans, MMWR (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 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, that 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

DRAFT--DO NOT QUOTE OR CITE

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 because 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., 1993a; 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 to 8 could be maintained in rats over a 22-week period with a loading dose of 25 $\mu\text{g/kg}$ followed by weekly maintenance doses of 5 $\mu\text{g/kg}$.

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 semilogarithmic plot in Figure 1-1 (log concentration versus time), which indicates apparent first-order elimination kinetics with

DRAFT--DO NOT QUOTE OR CITE

Figure 1-1

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

DRAFT--DO NOT QUOTE OR CITE

TABLE 1-5 (cont.)

TABLE 1-5 (cont.)						
Chemical	Species (Sex)	Dose	Tissue	Half-Life (days)	Remarks	Reference
2,3,7,8-TCDD	DBA/2J mice (F) Ah ^d /Ah ^d	0.5 µg/kg, i.p.	liver	11.9	Pool size (% of total dose):	Birnbaum, 1986
			adipose	11.8	20.2	
			skin	12.8	42.3	
					26.6	
2,3,7,8-TCDD	rhesus monkey (F)	25 ppt in diet	adipose	391 ± 88	Mean ± SE (n=7)	Bowman et al., 1989
OCDD	Fischer 344 rat (M)	50 µg/kg, i.v.	liver	84	Pool size (% of total dose):	Birnbaum and Couture, 1988
			adipose	38	72.7	
			skin	3	7.1	
				69	9.0, 1st component	
					0.3, 2nd component	
BDDs						
2,3,7,8-TBDD	Fischer 344 rat (M)	0.5 µg/kg, i.v. (0.001 µmol/kg)	liver	4.5	1st component	Kedderis et al., 1991a
				16.5	2nd component	
			adipose	57.8		
			skin	2.5	1st component	
				57.8	2nd component	
			muscle	1.6	1st component	
				26.7	2nd component	
			blood	18.2		
CDFs						
2,3,7,8-TCDF	Fischer 344 rat (M)	30.6 µg/kg, i.v. (0.1 µmol/kg)	liver	0.10	Pool size (% of total dose)	Birnbaum et al., 1980
				1.25	29.09 1st component	
			adipose	3.75	31.39 2nd component	
			skin	0.45	17.85	
				11.09	6.84 1st component	
			muscle	0.02	1.22 2nd component	
				0.72	24.85 1st component	
			blood	0.02	6.73 2nd component	
				1.14	1.31 1st component	
					0.89 2nd component	

TABLE 1-5 (cont.)

Chemical	Species (Sex)	Dose	Tissue	Half-Life (days)	Remarks	Reference
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
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., 1989a,b

TABLE 1-5 (cont.)

Chemical	Species (Sex)	Dose	Tissue	Half-Life (days)	Remarks	Reference
2,3,4,7,8-PeCDF	Fischer 344 rat (M)	34 µg/kg, i.v. (0.1 µmol/kg)	liver	193	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
			adipose	69		
			skin	0.62		
				1.23		
			muscle	0.04		
				0.51		
				9.84		
			blood	0.04		
				1.32		
				55		
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., 1989b
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., 1989b
PCBs						
3,3',4,4'-TCB	Sprague-Dawley rat (F)	5 mg/kg/day, p.o., for 21 days	liver	0.8	21-day exposure produced steady state with 300 ng/g in liver and 8 µg/g in adipose tissue.	Clarke et al., 1984
			adipose	2.5	Elimination was assessed over a 22-day postexposure period.	
3,3',4,4'-TCB	ICR mice (M)	8 mg/kg, p.o., every other day for 10 doses	liver	2.15	Steady-state tissue concentrations: 1.5 µg/g 19.2 µg/g 0.04 µg/mL	Clevenger et al., 1989
			adipose	2.60		
			serum	1.07		

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

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 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 rat, 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 have 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 those 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 twofold 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 $\mu\text{g/kg bw}$), which contained 0.12 μg 2,3,7,8-

TCDD/kg bw. Using the international toxicity equivalency (I-TE) factors (NATO, 1988; U.S. EPA, 1989), the total administered dose corresponded to 0.464 μ g 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 twofold 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-

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

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)
1,2,3,4,6,7,8-HpCDF	0.0186 ± 0.0072	37	21-152	-0.0140 ± 0.0137	∞^d	54- ∞^d
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

^aSource: Neubert et al., 1990.

^bAnimals 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.

^cCalculated from the time period: >6 weeks after injection.

^dCalculated half-life is apparently infinite. Data for OCDD and OCDF are unreliable due to delayed absorption.

^eNot detected in hepatic tissue 6 weeks after treatment; limits of detection used for calculation.

^fDue to interference.

NA = Not applicable.

DRAFT--DO NOT QUOTE OR CITE

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., 1989c). Additional short-term studies in rats provide evidence that the low tissue concentrations of non-2,3,7,8-substituted congeners, measured 1 week after exposure, were the result of rapid elimination, since these congeners were detected at higher levels in the liver 13 to 14 hours after exposure (Abraham et al., 1989d). 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 (Schechter et al., 1985, 1986; Ryan, 1986; Rappe et al., 1986; Beck et al., 1987; 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, 1989d). 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. (1989b) studied the hepatic disposition and elimination of CDFs 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 calculate half-lives accurately. 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 suggest that exposure to complex mixtures (see Table 1-6) may alter the tissue disposition of individual congeners.

Several studies have investigated potential pharmacokinetic interactions following exposure to two or more PCDDs, PCDFs, and PCBs (DeJongh et al., 1992, 1993a, 1993b). Interactive effects on the hepatic disposition of these compounds may in some cases explain, in part, the potentiation and antagonistic effects on CYP1A/1A2 activities observed with combined exposure to some of these compounds. In the case of 1,2,3,7,8-PeCDD and 2,4,5,2',4',5'-HxCB, no pharmacokinetic bases were found to explain the antagonistic effects of the combined exposure on CYP1A1/1A1 activities in mice (DeJongh et al., 1992). In a subsequent study the hepatic disposition of 1,2,3,7,8-PeCDD in mice was increased with combined exposures to 1,2,3,6,7,8-HxCDD, 2,3,4,7,8-PeCDF, and 2,4,5,2',4',5' HxCB (De Jongh et al., 1993b). Although there is evidence that PCDDs, PCDFs, and PCBs may influence each other's pharmacokinetics when administered in mixtures, this area needs investigation.

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 1-3,000 ng/kg bw. Greater than 97% of the administered 2,3,7,8-TCDD was absorbed at all doses, with the exception of the 3,000 ng/kg group where 84% of the dose was absorbed. Figure 1-2 illustrates the dose-dependent disposition of

DRAFT--DO NOT QUOTE OR CITE

Figure 1-2

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 disproportionately greater concentrations in the liver at high doses compared with low doses. Poiger et al. (1989a) observed a dose-related increase in distribution to the liver (% of dose/liver) and 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 $\mu\text{mol/kg}$ bw, respectively. In a related study, pretreatment of mice with 2,3,7,8-TCDD (5 or 15 $\mu\text{g/kg}$) produced a dose-related, enhanced hepatic accumulation of a subsequent oral dose of 2,3,7,8-TCDD (Curtis et al., 1990). Diliberto et al. (1993b) also observed a dose-dependent tissue distribution of 2,3,7,8-TCDD in female B6C3F1 mice. In all tissues except the liver, the relative percent of the total dose of TCDD decreased while it increased in the liver with higher doses. 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., 1989a; Leung et al., 1990b). 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.

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

^aSource: Abraham et al., 1988.

^bConcentrations were measured 7 days after injection.

ND = Not detectable; NA = not applicable.

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 8,100 ppt in fat and 24,000 ppt in the liver. Rats given 10 ng/kg/day had an average of 1,700 ppt 2,3,7,8-TCDD in the fat and 5,100 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 [¹⁴C]-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 about fivefold greater than that in fat. 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 because 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., 1990c). 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. (1990c) 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 disproportionately 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. (1989a,b) reported that TCDD and other Ah agonists (2,3,7,8-TCDF, β -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 (1×10^{-10} mol/kg) of [^{125} I]-2-iodo-3,7,8-trichlorodibenzo-*p*-dioxin to C57BL/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 (1×10^{-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 ED₅₀ ranging from 1.5 to 4.0×10^{-9} mol/kg. In congenic C57BL/6J (Ah^d/Ah^d) mice, which express the lower affinity Ah receptor, the ED₅₀ 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 (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., 1989a).

The following evidence reported by Poland et al. (1989b) 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 photoaffinity 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. (1989a,b) 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. (1989a,b) 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]-2-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.

A more recent study used pretreatment with isosafrole to investigate the role of CYP1A2 in the hepatic disposition of 2,3,7,8-TCDD (Kedderis et al., 1993a). Although isosafrole is an inducer of CYP1A2, it also has high affinity for the protein and thus is a selective inhibitor of CYP1A2. A greater than threefold decrease in the hepatic disposition of TCDD in isosafrole-pretreated rats supports the conclusion that TCDD is bound to CYP1A2 in the liver.

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 , for [^{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., 1989b). The induced microsomal binding species has an affinity about 10^4 times less than the Ah receptor but a pool size that is $\sim 2 \times 10^3$ 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; however, they do not exhibit dioxin-like activity. 2,2',4,4',5,5'-HxCB and -HxBB distribute primarily to adipose tissue, with partition coefficients (tissue/blood ratio) ranging from 300 to 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 to 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 that 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 (Kedderis et al., 1993b). 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 disposition observed at higher exposures of 2,3,7,8-TBDD (Kedderis et al., 1991b).

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 2,3,7,8-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., 1983b; 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 suggest 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, 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 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 compounds 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 β -glucuronidase, derivatized with diazomethane and separated into two compounds by high-performance liquid chromatography. 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. (1982) identified six metabolites in the bile of dogs that were given a lethal dose of [^3H]-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

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 Marrow, 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 Marrow, 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., 1983a

DRAFT--DO NOT QUOTE OR CITE

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	DBA/2J mice (M)	10 µg/kg, i.p.	100	100	82	1.2	24.4±1.0 ^d	NC	Gasiewicz et al., 1983a
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., 1983a
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., 1990b
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., 1990b
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

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	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
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., 1986b
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., 1986a
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

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				
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., 1991a
2,3,7,8-TBDD	Fischer 344 rat (M)	0.1 µmol/kg, iv	NA	100	80-90	9.2	0.6 17.8	Pool size (% of dose): 22.47 1st component 2.35 2nd component	Kedderis et al., 1991a
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

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-TCDF	DBA/2J mice (M)	0.1 μ mol/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 μ mol/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 μ g/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 μ mol/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 μ mol/kg, iv	~ 90	100	NA	12.8	0.92 3.32 1.26 17.32 1.12 6.30	Pool size (% of dose): <u>feces</u> : 57.79 1st component 6.92 2nd component <u>urine</u> : 2.68 1st component 0.16 2nd component <u>feces and urine</u> : 59.97 1st component 2.51 2nd component	Brewster and Birnbaum, 1988
2,3,4,7,8-PeCDF	Fischer 344 rat (M)	0.1 μ mol/kg, iv	NA	> 90	> 90	> 100	1.27 63.82	Pool size (% of dose): <u>feces</u> 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 μ mol/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

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

^bHalf-life for excretion estimates assume first-order elimination kinetics.

^c(mean $\pm$ SE).

^d(mean $\pm$ SD).

*n=1.

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

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 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 gas chromatography/mass spectroscopy. 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. 4-Hydroxy-2,3,7,8-TCDF was also the major TCDF metabolite formed by hepatic microsomes from TCDD-pretreated rats (Tai et al., 1993). 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 depend 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 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 dihydroxy-penta-CB and 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 hydroxy-tetra-CDF, dihydroxy-tri-CDF, dihydroxy-tetra-CDF, and 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 treated with this congener (Poiger et al., 1989a).

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., 1987b) 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, whereas 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 and 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 methylsulphonyl-TCB as major metabolites in the mouse fetus. Sulfur-containing metabolites of noncoplanar, nondioxin-like 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 sulfones are stable lipophilic metabolites formed by the mercapturic acid pathway. The toxicological significance of these metabolites of nondioxin-like PCBs remains generally unknown.

1.3.2. Toxicity of Metabolites

The discussion above indicates that 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 suggest that reactive epoxide intermediates may be formed. Poland and Glover (1970) investigated the *in vivo* binding of [1,6-³H]-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 per 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. (1982a) 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 $\mu\text{g}/\text{kg}$ of parent compound. Other groups of guinea pigs were given bile extract from 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 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 and thymus, liver, or spleen weights after exposure to a dose of $\leq 5000 \mu\text{g}/\text{kg}$ bw. 2-Hydroxy-3,7,8-TCDD induced hepatic microsomal AHH, EROD, and 4-chlorobiphenylhydroxylase activity at an exposure of 1,000 and 5,000 $\mu\text{g}/\text{kg}$ bw, whereas 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., 1982b).

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., 1987b). 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 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 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 because 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 that may occur at exposures 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 because 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., 1989a). The rates 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, 1993a) observed 10% of the dose of [^3H]-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 ($\sim 8\%$ of the dose) 24 hours after intravenous administration of 1 nmol/kg [^3H]-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 percent 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., 1993a). 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 [^3H]-congeners. Biliary excretion of the radiolabeled dose was quantitatively and qualitatively unaffected by pretreatment, despite a twofold increase in hepatic levels of [^3H] 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 $\mu\text{g/kg}$, intraperitoneal) excreting $9.7 \pm 1.9\%$ of the radiolabeled dose of 2,3,7,8-TCDD (200-300 $\mu\text{g/kg}$, per os) compared with $7.0 \pm 0.9\%$ 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 [^3H]-2,3,7,8-TCDD metabolites in a dog given a single oral dose of the [^3H]-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 $\mu\text{g/kg}$) 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 $\mu\text{mol/kg}$, 3 days earlier) significantly increased the biliary excretion of a subsequent dose of [^{14}C]-2,3,7,8-TCDF (McKinley et al., 1993). The naive rats excreted $5.69 \pm 2.35\%$ of the dose over the initial 8 hours, while the pretreated rats excreted $13.18 \pm 3.15\%$ of the [^{14}C]-2,3,7,8-TCDF. Similarly, pretreatment of rats with 2,3,4,7,8-PeCDF (500 $\mu\text{g/kg}$, per os, 3 days earlier) resulted in a twofold 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 or 1A2), which is also induced by 3,3',4,4'-TCB (Shimada and Sawabe, 1983; Mills et al., 1985; McKinley et al., 1993). 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 about fivefold 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 five- to sixfold, 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 were investigated in isolated rat hepatocytes incubated with [^3H]-2,3,7,8-TCDD at concentrations of 0.01, 0.1, and 1.0 μM (Olson et al., 1994). Lower 2,3,7,8-TCDD concentrations in the media result in concentrations in hepatocytes that 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 is similar to hepatic levels after in vivo exposure of rats at a dose of $\sim 10 \mu\text{g/kg}$. At 0.01 and 0.1 μM , the rate of metabolism of [^3H]-2,3,7,8-TCDD was similar in hepatocytes isolated from control and 2,3,7,8-TCDD-pretreated rats, whereas at 1.0 μM , [^3H]-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. Therefore, in vitro studies of the hepatic metabolism of TCDD (at 0.01 and 0.1 μM) are 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 [^3H]-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., 1994). 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., 1993).

TCDF metabolism was also investigated in rat liver, kidney, and lung microsomes in the presence and absence of selective chemical inhibitors and antibodies to CYP1A1 and CYP1A2 (Tai et al., 1993). Together, the results of this investigation indicate that CYP1A1 is the primary enzyme responsible for the metabolism of TCDF. TCDF was also metabolized by recombinant yeast microsomes expressing human CYP1A1 and reductase. However, based on EROD activity, a marker of CYP1A1, the relative rate of TCDF metabolism was about 100-fold greater in TCDD-induced rat liver microsomes than in yeast microsomes expressing human CYP1A1 and reductase (Tai et al., 1993). Although TCDF was metabolized by rat and human CYP1A1, the results indicated that there are marked quantitative differences in metabolism that suggest that TCDF will be more persistent in humans.

There are 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 2,120 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 rates of elimination of 2,3,7,8-TCDD and related compounds from major tissue depots (see Table 1-5) are 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., 1980). A similar excretion pattern was observed in mice, although there was significant strain variability (Gasiewicz et al., 1983a; 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]-2,3,7,8-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 [^3H]- or [^{14}C]-2,3,7,8-TCDD (see Table 1-8). While 15% to 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., 1983a; Olson et al., 1980). The daily presence of unchanged 2,3,7,8-TCDD in feces and its absence in bile suggest 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., 1991a). While direct intestinal elimination of the 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., 1989d). 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 that 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 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 than 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., 1980), 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 to 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 tissue distribution, metabolism, and excretion of 2,3,7,8-TCDD were also investigated in Han/Wistar and Long-Evans rats, which were, respectively, more resistant ($LD_{50} > 3000 \mu\text{g/kg}$) versus more susceptible ($LD_{50} \sim 10 \mu\text{g/kg}$) 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 about twofold greater adipose tissue stores than the C57BL/6J and B6D2F₁/J strains (Gasiewicz et al., 1983a). 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 congeneric mouse strains in which the congeneric 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 $\mu\text{g/kg/day}$ Monday through Friday for 7 weeks or a single dose of 1.0 $\mu\text{g/kg}$. In the single-dose study, no ^{14}C was excreted in the urine or expired air; in the repeated-dose study, however, 3% to 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., 1991a). 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., 1994). 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., 1993; Olson et al., 1994). Autoinduction of metabolism suggests 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) [^3H]-2,3,7,8-TCDD in 6 mL corn oil (see Table 1-8). The half-life for elimination was estimated to be 2,120 days. Table 1-9 summarizes additional half-life estimates for 2,3,7,8-TCDD and related compounds in

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*	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	4	4.7	Schechter et al., 1990a
				final 29 months	4	7.2	
				total 6 years	7	4.5	
1,2,3,4,7,8-HxCDF	Binghamton, New York, state office building	1	adipose tissue blood combined	initial 43 months	4	2.9	Schechter et al., 1990a
				final 29 months	4	4.4	
				total 6 years	7	4.0	
1,2,3,6,7,8-HxCDF	Binghamton, New York, state office building	1	adipose tissue blood combined	initial 43 months	4	3.5	Schechter et al., 1990a
				final 29 months	4	4.3	
				total 6 years	7	4.9	
1,2,3,4,6,7,8-HpCDF	Binghamton, New York, state office building	1	adipose tissue blood combined	initial 43 months	4	6.5	Schechter et al., 1990a
				final 29 months	4	4.1	
				total 6 years	7	6.8	
2,3,4,7,8-PeCDF	Yu-Cheng	4	blood	initial 2.9 years	2	1.3	Ryan and Masuda, 1989
		3		final 2.7 years	2	2.9	
		2		total 5.6 years	3	1.7	

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
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 and Masuda, 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 and Masuda, 1989
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

*95% confidence interval about the median of 5.8-9.6 years.

NA = Not applicable.

humans, based on serum and 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 to 10 years. The half-life values in Table 1-9 are rough estimates based on a small number of individuals and analysis at as few as two time points. Phillips (1989) discusses this issue. Estimates also assume a simple, single compartment, first-order elimination process.

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 $\mu\text{g}/\text{kg}$, whereas Yusho patients had levels of 0.1-5 $\mu\text{g}/\text{kg}$. In the Yu-Cheng individuals, half-lives for three CDFs were 2 to 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 occurred 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. (1990a) and Ryan and Masuda (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, lactation 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 (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 (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 levels from 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 (PB-PK) MODELS

Initially, PB-PK models were developed for 2,3,7,8-TCDD in C57BL/6J and DBA/2J mice (Leung et al., 1988), rats (Leung et al., 1990b) 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.

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

^aLeung et al., 1988.

^bLeung et al., 1990a.

^cKissel and Robarge, 1988.

^dLeung et al. (1990) modeled the disposition of [¹²⁵I]-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 μ mol/kg).

DRAFT--DO NOT QUOTE OR CITE

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. (1983a). 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 twofold greater total fat content in the DBA/2J strain. The authors suggested that strain difference in hepatic disposition was due to differing affinity of 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., 1990a). 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. (1978b). The model was not satisfactory for the 2-year feeding study of Kociba et al. (1978a), underpredicting the 2,3,7,8-TCDD concentration in the fat at the low dose ($0.001 \mu\text{g/kg/day}$) and overestimating the concentration achieved at the high dose ($0.1 \mu\text{g/kg/day}$). 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 to account for the differences in hepatic disposition at low ($0.01 \mu\text{g/kg}$) and high ($1.0 \mu\text{g/kg}$) 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., 1990b). Mice were pretreated with $0.1 \mu\text{mol/kg}$ 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 $0.17\text{--}0.38$, while the 2,3,7,8-TCDD pretreated mice had ratios of $2.0\text{--}6.1$. 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., 1989a). 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 to 20 nmol/liver and increased the rate constant for metabolism of free 2-iodo-3,7,8-TCDD from 1 to 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 that 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., 1993; Olson et al., 1994).

Andersen et al. (1993) recently described 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. (1993) recently developed a mechanistic model of the effects of dioxin on gene 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-TCDD.

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., 1990c; Ryan et al., 1987), although this is not the case in rodents (Leung et al., 1988, 1990a). 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 that 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. (1993b; Kedderis, 1993) 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., 1990a; Poland et al., 1989b) and uses 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., 1993). 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 $\mu\text{mol/kg}$ (30.6 $\mu\text{g/kg}$). 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., 1993; Olson et al., 1994). 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.

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
K _m (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

^aUrinary clearance/biliary clearance.

DRAFT--DO NOT QUOTE OR CITE

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 compartments, tissue-specific binding 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 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 are 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} ($\mu\text{g}/\text{kg}$)—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, K_d 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 is 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. Second is the assumption 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 $\mu\text{g/kg}$) and [^{14}C]-2,3,7,8-TCDF (800 $\mu\text{g/kg}$) 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 those of males of the same strain (Gasiewicz et al., 1983a; 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 was 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 was 1.3, 1.8, and 1.8 times faster than that observed for males. Elimination data from pregnant mice were based on only three time

points (gestation days 12, 13, and 14) and thus represent 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 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 $\mu\text{g/kg}$) and [^{14}C]-2,3,7,8-TCDF (800 $\mu\text{g/kg}$) 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 with 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 $\mu\text{g/kg}$ 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% to 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% to 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 to 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 $\mu\text{g/kg}$ 2,3,7,8-TCDD on gestation day 7 or 10 or 5 $\mu\text{g/kg/day}$, 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 $0.038 \pm 0.011\%$ (0.36 ng/g), $0.096 \pm 0.027\%$ (0.92 ng/g), and $0.12 \pm 0.05\%$ (1.1 ng/g) of the dose (mean $\pm$ 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 $\mu\text{g}/\text{kg}$. 2,3,7,8-TCDD was detected in the gestation day-11 embryo at 3 hours postexposure and was equally distributed between the embryonic head and body. At 72 hours postexposure, 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 in 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 $\mu\text{g}/\text{kg}$ 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 $\sim 2\%$ 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 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).

DRAFT--DO NOT QUOTE OR CITE

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. (1987b) 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 ($\sim 0.15\%$ 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 threefold 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

DRAFT--DO NOT QUOTE OR CITE

2,3,7,8-TCDD in the 33-day-old infant was $\sim 0.9\%$ of the dose/g tissue. Transfer of hepta- and octa-CDDs and CDFs to the suckled infant was rather low, only $\sim 10\%$ 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 2,494 pg I-TE/g, while the offspring liver contained 2,022 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 $\pm$ 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 and 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 to 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% to 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 to 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 postpartum. 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 $17.7 \pm 2.7\%$ (mean $\pm$ SD) to $5.6 \pm 2.5\%$ 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 $22.2 \pm 0.2\%$ to $14.7 \pm 3.8\%$ 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/cm²) 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., 1993). 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.

1.6. REFERENCES

Abbott, B.D.; Diliberto, J.J.; Birnbaum, L.S. (1989) 2,3,7,8-TCDD alters embryonic palatal medial epithelial cell differentiation in vitro. *Toxicol. Appl. Pharmacol.* 100: 119-131.

Abdel-Hamid, F.M.; Moore, J.A.; Matthews, H.B. (1981) Comparative study of 3,4,3',4'-tetrachlorobiphenyl in male and female rats and female monkeys. *J. Toxicol. Environ. Health.* 7: 181-191.

Abraham, K.; Krowke, R.; Neubert, D. (1988) Pharmacokinetics and biological activity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 1. Dose-dependent tissue distribution and induction of hepatic ethoxyresorufin o-deethylase in rats following a single injection. *Arch. Toxicol.* 62: 359-368.

Abraham, K.; Krowke, R.; Neubert, D. (1989a) Absorption of TCDD following parenteral application in rats using various vehicles. *Chemosphere* 19(1-6): 893-898.

Abraham, K.; Weberrub, U.; Wiesmuller, T.; Hagenmaier, H.; Krowke, R.; Neubert, D. (1989b) Comparative studies on absorption and distribution in the liver and adipose tissue of PCDDs and PCDFs in rats and marmoset monkeys. *Chemosphere* 19(1-6): 887-892.

Abraham, K.; Wiesmuller, T.; Brunner, H.; Krowke, R.; Hagenmaier, H.; Neubert, D. (1989c) Absorption and tissue distribution of various polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs and PCDFs) in the rat. *Arch. Toxicol.* 63: 193-202.

Abraham, K.; Wiesmuller, T.; Brunner, H.; Krowke, R.; Hagenmaier, H.; Neubert, D. (1989d) Elimination of various polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs and PCDFs) in rat faeces. *Arch. Toxicol.* 63: 75-78.

Abraham, K.; Wiesmuller, T.; Hagenmaier, H.; Neubert, D. (1990) Distribution of PCDDs and PCDFs in various tissues of marmoset monkeys. *Chemosphere* 20(7-9): 1971-1078.

Ademola, J.I.; Wester, R.C.; Maibach, H.I. (1993) Absorption and metabolism of 2-chloro-2,6-diethyl-N-(butoxymethyl)acetanilide (butachlor) in human skin in vitro. *Toxicol. Appl. Pharmacol.* 121(1): 78-86.

Ahlborg, U.G.; Hakansson, H.; Lindstrom, G.; Rappe, C. (1990) Studies on the retention of individual polychlorinated dibenzofurans (PCDFs) in the liver of different species. *Chemosphere* 20(7-9): 1235-1240.

Albro, P.W.; Fishbein, L. (1972) Intestinal absorption of polychlorinated biphenyls in rats. *Bull. Environ. Contam. Toxicol.* 8(1): 26-31.

DRAFT--DO NOT QUOTE OR CITE

Allen, J.R.; Van Miller, J.P.; Norback, D.H. (1975) Tissue distribution excretion and biological effects of [¹⁴C]tetrachlorodibenzo-*p*-dioxin in rats. Food Cosmet. Toxicol. 13(5): 501-505.

Andersen, M.E.; Mills, J.J.; Garga, M.L.; et al. (1993) Modelling receptor-mediated processes with dioxin: implications for pharmacokinetics and risk assessment. Risk Anal. (in press).

Anderson, M.W.; Eling, T.E.; Lutz, R.J.; Dedrick, R.L.; Matthews, H.B. (1977) The construction of a pharmacokinetic model for the disposition of polychlorinated biphenyls in the rat. Clin. Pharm. Therapeut. 20: 765-773.

Anderson, Y.B.; Jackson, J.A.; Birnbaum, L.S. (1993) Maturational changes in dermal absorption of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in Fisher 344 rats. Toxicol. Appl. Pharmacol. 119: 214-220.

Appelgren, L.E.; Brandt, I.; Brittelos, E.B.; Gillner, M.; Gustafsson, S.A. (1983) Autoradiography of 2,3,7,8-tetrachloro-¹⁴Cl-dibenzo-*p*-dioxin TCDD: accumulation in the nasal mucosa. Chemosphere 12(4/5): 545-548.

Arstila, A.U.; Reggiani, G.; Sorvari, T.E.; Raisanen, S.; Wipf, H.K. (1981) Elimination of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in goat milk. Toxicol. Lett. 9: 215-219.

Banks, Y.B.; Birnbaum, L.S. (1991a) Absorption of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) after low dose dermal exposure. Toxicol. Appl. Pharmacol. 107: 302-310.

Banks, Y.B.; Birnbaum, L.S. (1991b) Kinetics of 2,3,7,8-tetrachlorodibenzofuran (TCDF) absorption after low dose dermal exposure. Toxicologist 11: 270.

Banks, Y.B.; Brewster, D.W.; Birnbaum, L.S. (1990) Age-related changes in dermal absorption of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,3,4,7,8-pentachlorodibenzofuran. Fund. Appl. Toxicol. 15: 163-173.

Baughman, R.W. (1975) Tetrachlorodibenzo-*p*-dioxins in the environment. High resolution mass spectrometry at the picogram level. Harvard University. NTIS PB75-22939.

Beck, H.; Eckart, K.; Mathar, W.; Wittkowski, R. (1987) Isomerenspezifische Bestimmung von PCDD und PCDF in Human- und Lebensmittelproben. VDI Berichte. 634: 359-382.

Beck, H.; Eckart, K.; Mathar, W.; Wiltkowski, R. (1989) Levels of PCDDs and PCDFs in adipose tissue of occupationally exposed workers. Chemosphere 18: 507-516.

DRAFT--DO NOT QUOTE OR CITE

- Beck, H.; Drob, A.; Kleeman, W.J.; Mathar, W. (1990) PCDD and PCDF concentrations in different organs from infants. *Chemosphere* 20(7-9): 903-910.
- Becker, M.M.; Gamble, W. (1982) Determination of the binding of 2,4,5,2',4',5'-hexachlorobiphenyl by low density lipoprotein and bovine serum albumin. *J. Toxicol. Environ. Health* 9: 225-234.
- Bergman, A.; Brandt, I.; Jansson, B. (1979) Accumulation of methylsulfonyl derivatives of some bronchial-seeking polychlorinated biphenyls in the respiratory tract of mice. *Toxicol. Appl. Pharmacol.* 48: 213-220.
- Bickel, M.H.; Muehlebach, S. (1980) Pharmacokinetics and ecodisposition of polyhalogenated hydrocarbons: aspects and concepts. *Drug Metab. Rev.* 11(2): 149-190.
- Birnbaum, L.S. (1983) Distribution and excretion of 2,3,6,2',3',6'- and 2,4,5,2',4',5'-hexachlorobiphenyl in senescent rats. *Toxicol. Appl. Pharmacol.* 70: 262-272.
- Birnbaum, L.S. (1985) The role of structure in the disposition of halogenated aromatic xenobiotics. *Environ. Health. Perspect.* 61: 11-20.
- Birnbaum, L.S. (1986) Distribution and excretion of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in congenic strains of mice which differ at the Ah locus. *Drug Metab. Dispos.* 14(1): 34-40.
- Birnbaum, L.S.; Couture, L.A. (1988) Disposition of octachlorodibenzo-*p*-dioxin (OCDD) in male rats. *Toxicol. Appl. Pharmacol.* 93: 22-30.
- Birnbaum, L.S.; Decad, G.M.; Matthews, H.B. (1980) Disposition and excretion of 2,3,7,8-tetrachlorodibenzofuran in the rat. *Toxicol. Appl. Pharmacol.* 55: 342-352.
- Birnbaum, L.S.; Decad, G.M.; Matthews, H.B.; McConnell, E.E. (1981) Fate of 2,3,7,8-tetrachlorodibenzofuran in the monkey. *Toxicol. Appl. Pharmacol.* 57: 189-196.
- Bonaccorsi, A.; diDomenico, A.; Fanelli, R.; et al. (1984) The influence of soil particle adsorption on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin biological uptake in the rabbit. *Arch. Toxicol. Suppl.* 7: 431-434.
- Bowman, R.E.; Schantz, S.L.; Weerasinghe, N.C.A.; et al. (1987) Clearance of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) from body fat of rhesus monkeys following chronic exposure. *Toxicologist* 7: 158.

DRAFT--DO NOT QUOTE OR CITE

Bowman, R.E.; Schantz, S.L.; Weerasinghe, N.C.A.; Gross, M.L.; Barsotti, D.A. (1989) Chronic dietary intake of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at 5 or 25 parts per trillion in the monkey: TCDD kinetics and dose-effect estimate of reproductive toxicity. *Chemosphere* 18(1-6): 243-252.

Bowman, R.E.; Tong, H.Y.; Gross, M.L.; Monson, S.J.; Weerasinghe, N.C.A. (1990) Controlled exposure of female rhesus monkeys to 2,3,7,8-TCDD: concentrations of TCDD in fat of offspring, and its decline overtime. *Chemosphere* 20(7-9): 1199-1202.

Brandt, I.; Darnerud, P.O.; Bergman, A.; Larsson, Y. (1982) Metabolism of 2,4',5-trichlorobiphenyl: enrichment of hydroxylated and methyl sulphone metabolites in the uterine luminal fluid of pregnant mice. *Chem.-Biol. Interact.* 40: 45-56.

Brewster, D.W.; Birnbaum, L.S. (1987) Disposition and excretion of 2,3,4,7,8-pentachlorodibenzofuran in the rat. *Toxicol. Appl. Pharmacol.* 90: 243-252.

Brewster, D.W.; Birnbaum, L.S. (1988) Disposition of 1,2,3,7,8-pentachlorodibenzofuran in the rat. *Toxicol. Appl. Pharmacol.* 95: 490-498.

Brewster, D.W.; Elwell, M.R.; Birnbaum, L.S. (1988) Toxicity and disposition of 2,3,4,7,8-pentachlorodibenzofuran (4PeCDF) in the rhesus monkey (*Macaca mulatta*). *Toxicol. Appl. Pharmacol.* 93: 231-246.

Brewster, D.W.; Banks, Y.B.; Clark, A-M.; Birnbaum, L.S. (1989) Comparative dermal absorption of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and three polychlorinated dibenzofurans. *Toxicol. Appl. Pharmacol.* 97: 156-166.

Brouwer, A.; van den Berg, K.J.; Kukler, A. (1985) Time and dose responses of the reduction in retinoid concentrations in C57BL/Rij and DBA/2 mice induced by 3,4,3',4'-tetrachlorobiphenyl. *Toxicol. Appl. Pharmacol.* 78: 180-189.

Brunner, H.; Wiesmuller, T.; Hagenmaier, H.; Abraham, K.; Krowke, R.; Neubert, D. (1989) Distribution of PCDDs and PCDFs in rat tissues following various routes of administration. *Chemosphere* 19(1-6): 907-912.

Brunstrom, B.; Darnerud, P.O. (1983) Toxicity and distribution in chick embryos of 3,3',4,4'-tetrachlorobiphenyl injected into the eggs. *Toxicology* 27: 103-110.

Burka, L.T.; McGown, S.R.; Tomer, K.B. (1990) Identification of the biliary metabolites of 2,3,7,8-tetrachlorodibenzofuran in the rat. *Chemosphere* 21(10-11): 1231-1242.

DRAFT--DO NOT QUOTE OR CITE

- Byard, J.L. (1987) The toxicological significance of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related compounds in human adipose tissue. *J. Toxicol. Environ. Health.* 22: 381-403.
- Carrier, G.; Brodeur, J. (1991) Non-linear toxicokinetic behavior of TCDD-like halogenated polycyclic aromatic hydrocarbons (H-PAH) in various species. *Toxicologist* 11: 237.
- CDC (Centers for Disease Control). (1988) Serum 2,3,7,8-tetrachlorodibenzo-*p*-dioxin levels in Air Force health study participants—preliminary report. *J. Am. Med. Assoc.* 259(24): 3533-3535.
- Chahoud, I.; Krowke, R.; Bochert, G.; Burkle, B.; Neubert, D. (1991) Reproductive toxicity and toxicokinetics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 2. Problem of paternally-mediated abnormalities in the progeny of rat. *Arch. Toxicol.* 65: 27-31.
- Chen, P.H.; Luo, M.L.; Wong, C.K.; Chen, C.J. (1982) Comparative rates of elimination of some individual polychlorinated biphenyls from the blood of PCB-poisoned patients in Taiwan. *Food Cosmet. Toxicol.* 20: 417-425.
- Chen, P.H.; Wong, C.; Rappe, C.; Nygren, M. (1985) Polychlorinated biphenyls, dibenzofurans and quaterphenyls in toxic rice-bran oil and in the blood and tissues of patients with PCB poisoning (Yu-Cheng) in Taiwan. *Environ. Health Perspect.* 59: 59-65.
- Clark, G.; Tritscher, A.; McCoy, Z.; et al. (1991) Dose-response relationships for chronic exposure to 2,3,7,8-TCDD in a rat liver tumor promotion model: 1. Relationships of TCDD tissue concentrations to serum clinical chemistry, cell proliferation, and preneoplastic foci. In: *Proceedings of 11th international symposium on chlorinated dioxins and related compounds, Dioxin '91; September 23-27, 1991; Research Triangle Park, NC; p. 170.*
- Clarke, D.W.; Brien, J.F.; Nakatsu, K.; Taub, H.; Racz, W.J.; Marks, G.S. (1983) Gas-liquid chromatographic determination of the distribution of 3,3',4,4'-tetrachlorobiphenyl in the adult female rat following short-term oral administration. *Can. J. Physiol. Pharmacol.* 61: 1093-1100.
- Clarke, D.W.; Brien, J.F.; Racz, W.J.; Nakatsu, K.; Marks, G.S. (1984) The disposition and the liver and thymus gland toxicity of 3,3',4,4'-tetrachlorobiphenyl in the female rat. *Can. J. Physiol. Pharmacol.* 62: 1253-1260.
- Clevenger, M.A.; Roberts, S.M.; Lattin, D.L.; Harbison, R.D.; James, R.C. (1989) The pharmacokinetics of 2,2',5,5'-tetrachlorobiphenyl and 3,3',4,4'-tetrachlorobiphenyl and its relationship to toxicity. *Toxicol. Appl. Pharmacol.* 100: 315-327.

DRAFT--DO NOT QUOTE OR CITE

Coccia, P.; Croci, T.; Manara, L. (1981) Less TCDD persists in liver 2 weeks after a single dose to mice fed chow with added charcoal or choleic acid. *Br. J. Pharmacol.* 72: 181P.

Couture, L.A.; Harris, M.W.; Birnbaum, L.S. (1990) Characterization of the peak period of sensitivity for the induction of hydronephrosis in C57BL/6N mice following exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Fund. Appl. Toxicol.* 15: 142-150.

Curtis, L.R.; Kerkvliet, N.I.; Baecher-Steppan, L.; Carpenter, H.M. (1990) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin pretreatment of female mice altered tissue distribution but not hepatic metabolism of a subsequent dose. *Fund. Appl. Toxicol.* 14: 523-531.

Darnerud, P.O.; Brandt, I.; Klasson-Wehler, E.; et al. (1986) 3,3',4,4'-tetrachloro[¹⁴C]biphenyl in pregnant mice: enrichment of phenol and methyl sulphone metabolites in late gestational fetuses. *Xenobiotica* 16(4): 295-306.

Decad, G.M.; Birnbaum, L.S.; Matthews, H.B. (1981a) 2,3,7,8-tetrachlorodibenzofuran tissue distribution and excretion in guinea pigs. *Toxicol. Appl. Pharmacol.* 57: 231-240.

Decad, G.M.; Birnbaum, L.S.; Matthews, H.B. (1981b) Distribution and excretion of 2,3,7,8-tetrachlorodibenzofuran in C57BL/6J and DBA/2J mice. *Toxicol. Appl. Pharmacol.* 59: 564-573.

De Jongh, J.; Wondergem, F.; Seinen, W.; Van den Berg, M. (1992) Absence of interactions on hepatic retention and 7-ethoxyresorufin-O-deethylation activity after co-administration of 1,2,3,8-pentachlorodibenzo-*p*-dioxin and 2,4,5,2',4',5'-hexachlorobiphenyl. *Toxicology* 75: 21-28.

De Jongh, J.; Wondergem, F.; Seinen, W.; Van den Berg, M. (1993a) Toxicokinetic interactions between chlorinated aromatic hydrocarbons in the liver of the C57BL/6J mouse: I. Polychlorinated biphenyls (PCBs). *Arch. Toxicol.* 67: 453-460.

De Jongh, J.; Nieboer, R.; Schröders, I.; Seinen, W.; Van den Berg, M. (1993b) Toxicokinetic mixture interactions between chlorinated aromatic hydrocarbons in the liver of the C57BL/6J mouse: 2. Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls. *Arch. Toxicol.* 67: 598-604.

Diliberto, J.J.; Kedderis, L.B.; Birnbaum, L.S. (1990) Absorption of 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD) in male rats. *Toxicologist* 10: 54.

Diliberto, J.J.; Jackson, J.A.; Birnbaum, L.S. (1992) Disposition and absorption of intratracheal, oral, and intravenous ³H-TCDD in male Fischer rats. *Toxicologist* 12: 79.

DRAFT--DO NOT QUOTE OR CITE

- Diliberto, J.J.; Kedderis, L.B.; Jackson, J.A.; Birnbaum, L.S. (1993a) Effects of dose and routes of exposure on the disposition of 2,3,7,8-[³H]tetrabromodibenzo-p-dioxin (TBDD) in the rat. *Toxicol. Appl. Pharmacol.* 120: 315-326.
- Diliberto, J.J.; Akubue, P.I.; Jackson, J.A.; Luebke, R.W.; Copeland, C.B.; Birnbaum, L.S. (1993b) Dose-dependent tissue distribution of 2,3,7,8-TCDD in mice. *Toxicologist* 13: 195.
- Durham, S.K.; Brouwer, A. (1989a) 3,4,3',4'-tetrachlorobiphenyl-induced effects in the rat liver. I. Serum and hepatic retinoid reduction and morphologic changes. *Toxicol. Path.* 17(3): 536-544.
- Durham, S.K.; Brouwer, A. (1989b) 3,4,3',4'-tetrachlorobiphenyl-induced effects in the rat liver. II. Electron microscopic autoradiographic localization of ³H-TCB. *Toxicol. Path.* 17(4): 782-788.
- Durham, S.K.; Brouwer, A. (1990) 3,4,3',4'-tetrachlorobiphenyl distribution and induced effects in the rat adrenal gland. Localization in the zona fasciculata. *Lab. Invest.* 62(2): 232-239.
- Ebner, K.V.; Braselton, W.E., Jr. (1987) Structural and chemical requirements for hydroxychlorobiphenyls to uncouple rat liver mitochondria and potentiation of uncoupling with aroclor 1254. *Chem. Biol. Interact.* 63: 139-155.
- Ecobichon, D.J.; Hidvegi, S.; Comeau, A.M.; Cameron, P.H. (1983) Transplacental and milk transfer of polybrominated biphenyls to perinatal guinea pigs from treated dams. *Toxicology* 28: 51-63.
- Eyster, J.T.; Humphrey, H.E.B.; Kimbrough, R.D. (1983) Partitioning of polybrominated biphenyls (PBBs) in serum, adipose tissue, breast milk, placenta, cord blood, biliary fluid, and feces. *Arch. Environ. Health* 38(1): 47-53.
- Fachetti, A.; Fornari, A.; Montagna, M. (1980) Distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the tissues of a person exposed to the toxic cloud at Seveso (Italy). *Adv. Mass Spectrom.* 8B: 1405-1414.
- Fries, G.F.; Marrow, G.S. (1975) Retention and excretion of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by rats. *J. Agric. Food Chem.* 23(2): 265-269.
- Furst, P.; Meemken, H.A.; Groebel, W. (1986) Determination of polychlorinated dibenzodioxins and dibenzofurans in human milk. *Chemosphere* 15: 1977-1980.

- Furst, P.; Meemken, H.-A.; Kruger, C.H.R.; Groebel, W. (1987) Polychlorinated dibenzodioxins and dibenzofurans in human milk samples from Western Germany. *Chemosphere* 16(8/9): 1983-1988.
- Furst, P.; Kruger, C.; Meemken, H.-A.; Groebel, W. (1989) PCDD and PCDF levels in human milk—dependence on the period of lactation. *Chemosphere* 18(1-6): 439-444.
- Gallenberg, L.A.; Ring, B.J.; Vodick, M.J. (1990) The influence of time of maternal exposure to 2,4,5,2',4',5'-hexachlorobiphenyl on its accumulation in their nursing offspring. *Toxicol. Appl. Pharmacol.* 104: 1-8.
- Gallo, M.A.; Rahman, M.S.; Zatz, J.L.; Meeker, R.J. (1992) In vitro dermal uptake of 2,3,7,8-TCDD in hairless mouse and human skin from laboratory-contaminated soils. *Toxicologist* 12: 80.
- Gasiewicz, T.A.; Neal, R.A. (1979) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin tissue distribution, excretion, and effects on clinical chemical parameters in guinea pigs. *Toxicol. Appl. Pharmacol.* 51(2): 329-340.
- Gasiewicz, T.A.; Geiger, L.E.; Rucci, G.; Neal, R.A. (1983a) Distribution, excretion, and metabolism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in C57BL/6J, DBA/2J, and B6D2F1/J mice. *Drug Metab. Dispos.* 11(5): 397-403.
- Gasiewicz, T.A.; Olson, J.R.; Geiger, L.E.; Neal, R.A. (1983b) Absorption, distribution and metabolism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in experimental animals. In: Tucker, R.E.; Young, A.L.; Gray, A.P., eds. *Human and environmental risks of chlorinated dioxins and related compounds*. New York, NY: Plenum Press; pp. 495-525.
- Gehring, P.J. (1976) Pharmacokinetics: first order or zero order? *Food Cosmet. Toxicol.* 14: 654-654.
- Geyer, H.J.; Scheunert, I.; Filser, J.G.; Korte, F. (1986) Bioconcentration potential (BCP) of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) in terrestrial organisms including humans. *Chemosphere* 15(9-12): 1495-1502.
- Geyer, H.J.; Scheunert, I.; Korte, F. (1987) Correlation between the bioconcentration potential of organic environmental chemicals in humans and their n-octanol/water partition coefficients. *Chemosphere* 16(1): 239-252.
- Gillette, D.M.; Corey, R.D.; Helferich, W.G.; et al. (1987) Comparative toxicology of tetrachlorobiphenyls in mink and rats. *Fund. Appl. Toxicol.* 8: 5-14.

DRAFT--DO NOT QUOTE OR CITE

Gillner, M.; Brittebo, E.B.; Brandt, I.; Soderkvist, P.; Applegren, L-E.; Gustafsson, J-A. (1987) Uptake and specific binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the olfactory mucosa of mice and rats. *Cancer Res.* 47: 4150-4159.

Gochfeld, M.; Nygren, M.; Hansson, M.; et al. (1989) Correlation of adipose and blood levels of several dioxin and dibenzofuran congeners in agent orange exposed Viet Nam veterans. *Chemosphere* 18(1-6): 517-524.

Gorski, T.; Konopka, L.; Brodzki, M. (1984) Persistence of some polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans of pentachlorophenol in human adipose tissue. *Roczn. Pzh. T.* 35(4): 297-301.

Graham, M.; Hileman, F.D.; Orth, R.G.; Wendling, J.M.; Wilson, J.W. (1986) Chlorocarbons in adipose tissue from a Missouri population. *Chemosphere* 15: 1595-1600.

Guenthner, T.M.; Fysh, J.M.; Nebert, D.W. (1979) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: covalent binding of reactive metabolic intermediates principally to protein in vitro. *Pharmacology* 19: 12-22.

Guo, Y.L.; Emmett, E.A.; Pellizzari, E.D.; Rohde, C.A. (1987) Influence of serum cholesterol and albumin on partitioning of PCB congeners between human serum and adipose tissue. *Toxicol. Appl. Pharmacol.* 87: 48-56.

Hagenmaier, H.; Wiesmuller, T.; Golor, G.; Krowke, R.; Helge, H.; Neubert, D. (1990) Transfer of various polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs and PCDFs) via placenta and through milk in a marmoset monkey. *Arch. Toxicol.* 64: 601-615.

Hakansson, H.; Hanberg, A.; Ahlborg, U.G. (1989) The distribution of ¹⁴C-2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) between parenchymal and non-parenchymal rat hepatic cells and its effect on the vitamin A content of these cells. *Chemosphere* 18(1-6): 307-312.

Haraguchi, K.; Kurocki, H.; Masuda, Y. (1986) Capillary gas chromatographic analysis of methylsulphone metabolites of polychlorinated biphenyls retained in human tissues. *J. Chromatog.* 361: 239-252.

Haraguchi, K.; Kurocki, H.; Masuda, Y. (1989) Polychlorinated biphenyl methylsulfone congeners in human tissues: identification of methylsulfonyl dichlorobiphenyls. *Chemosphere* 18(1-6): 477-484.

Hayabuchi, H.; Ikeda, M.; Yoshimura, T.; Masuda, Y. (1981) Relationship between the consumption of toxic rice oil and the long-term concentration of polychlorinated biphenyls in the blood of Yusho patients. *Food Cosmet. Toxicol.* 19: 53-55.

DRAFT--DO NOT QUOTE OR CITE

Hayward, D.G.; Charles, J.M.; Voss de Bettancourt, C.; Stephens, S.E.; Stephens, T.D. (1989) PCDD and PCDF in breast milk as correlated with fish consumption in southern California. *Chemosphere* 18(1-6) 455-468.

Hebert, C.D.; Birnbaum, L.S. (1987) The influence of aging on intestinal absorption of TCDD in rats. *Toxicol. Lett.* 37: 47-55.

Henderson, L.O.; Patterson, D.G., Jr. (1988) Distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in human whole blood and its association with, and extractability from lipoproteins. *Bull. Environ. Contam. Toxicol.* 40: 604-611.

Hiles, R.A.; Bruce, R.D. (1976) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin elimination in the rat: first order or zero order? *Food Cosmet. Toxicol.* 14: 599-600.

Huetter, R.; Philippi, M. (1982) Studies on microbial metabolism of TCDD under laboratory conditions. *Pergamon Ser. Environ. Sci.* 5: 87-93.

Ioannou, Y.M.; Birnbaum, L.S.; Matthews, H.B. (1983) Toxicity and distribution of 2,3,7,8-tetrachlorodibenzofuran in male guinea pigs. *J. Toxicol. Environ. Health.* 12: 541-553.

Ivens, I.; Neupert, M.; Loser, E.; Thies, J. (1990) Storage and elimination of 2,3,7,8-tetrabromodibenzo-*p*-dioxin in liver and adipose tissue of the rat. *Chemosphere* 20(7-9): 1209-1214.

Jackson, J.A.; Diliberto, J.J.; Birnbaum, L.S. (1993) Estimation of octanol-water partition coefficients and correlation with dermal absorption for several polyhalogenated aromatic hydrocarbons. *Fund. Appl. Toxicol.* 21:334-344.

Jensen, A.A. (1987) Polychlorobiphenyls (PCBs), polychlorodibenzo-*p*-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs) in human milk, blood and adipose tissue. *Sci. Total Environ.* 64: 259-293.

Jondorf, W.R.; Wyss, P.A.; Muhlebach, S.; Bickel, M.H. (1983) Disposition of 2,2',4,4',5,5'-hexachlorobiphenyl (6-CB) in rats with decreasing adipose tissue mass. II. Effects of restricting food intake before and after 6-CB administration. *Drug Metab. Dispos.* 11(6): 597-601.

Kahn, P.C.; Gochfeld, M.; Nygren, M.; et al. (1988) Dioxins and dibenzofurans in blood and adipose tissue of agent orange-exposed Vietnam veterans and matched controls. *J. Am. Med. Assoc.* 259(11): 1661-1667.

DRAFT--DO NOT QUOTE OR CITE

Kamimura, H.; Koga, N.; Oguri, K.; Yoshimura, H.; Honda, Y.; Nakano, M. (1988) Enhanced faecal excretion of 2,3,4,7,8-pentachlorodibenzofuran in rats by a long-term treatment with activated charcoal beads. *Xenobiotica* 18(5): 585-592.

Kaminsky, L.S.; DeCaprio, A.P.; Gierthy, J.F.; Silkworth, J.B.; Tumasonis, C. (1985) The role of environmental matrices and experimental vehicles in chlorinated dibenzodioxin and dibenzofuran toxicity. *Chemosphere* 14(6/7): 685-695.

Kannan, N.; Tanabe, S.; Tatsukawa, R. (1988) Potentially hazardous residues of non-ortho chlorine substituted coplanar PCBs in human adipose tissue. *Arch. Environ. Health.* 43(1): 11-14.

Kedderis, L.B. (1993) Biologically-based models of dioxin pharmacokinetics. *HERL Symposium*.

Kedderis, L.B.; Diliberto, J.J.; Birnbaum, L.S. (1991a) Disposition and excretion of intravenous 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD) in rats. *Toxicol. Appl. Pharmacol.* 108: 397-406.

Kedderis, L.B.; Diliberto, J.J.; Linko, P.; Goldstein, J.A.; Birnbaum, L.S. (1991b) Disposition of TBDD and TCDD in the rat: biliary excretion and induction of cytochromes P4501A1 and P4501A2. *Toxicol. Appl. Pharmacol.* 111: 163-172.

Kedderis, L.B.; Andersen, M.E.; Birnbaum, L.S. (1993a) Effect of dose, time, and pretreatment on the biliary excretion and tissue distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. *Fund. Appl. Toxicol.* 21: 405-411.

Kedderis, L.B.; Mills, J.J.; Andersen, M.E.; Birnbaum, L.S. (1993b) A physiologically-based pharmacokinetic model for 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD) in the rat: Tissue distribution and CYP1A induction. *Toxicol. Appl. Pharmacol.* 121: 87-98.

King, F.G.; Dedrick, R.L.; Collins, J.M.; Matthews, H.B.; Birnbaum, L.S. (1983) Physiological model for the pharmacokinetics of 2,3,7,8-tetrachlorodibenzofuran in several species. *Toxicol. Appl. Pharmacol.* 67: 390-400.

Kissel, J.C.; Robarge, G.M. (1988) Assessing the elimination of 2,3,7,8-TCDD from humans with a physiologically based pharmacokinetic model. *Chemosphere* 17(10): 2017-2027.

Kleeman, J.M.; Olson, J.R.; Chen, S.M.; Peterson, R.E. (1986a) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin metabolism and disposition in yellow perch. *Toxicol. Appl. Pharmacol.* 83: 402-411.

DRAFT--DO NOT QUOTE OR CITE

Kleeman, J.M.; Olson, J.R.; Chen, S.M.; Peterson, R.E. (1986b) Metabolism and disposition of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rainbow trout. Toxicol. Appl. Pharmacol. 83: 391-401.

Kleeman, J.M.; Olson, J.R.; Peterson, R.E. (1988) Species differences in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity and biotransformation in fish. Fund. Appl. Toxicol. 10: 206-213.

Kociba, R.J.; Keeler, P.A.; Park, C.N.; Gehring, P.J. (1976) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin results of a 13-week oral toxicity study in rats. Toxicol. Appl. Pharmacol. 35: 553-574.

Kociba, R.J.; Keyes, D.G.; Beyer, J.E.; et al. (1978a) Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. Toxicol. Appl. Pharmacol. 46(2): 279-303.

Kociba, R.J.; Keyes, D.G.; Beyer, J.E.; Carreon, R.M. (1978b) Toxicologic studies of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in rats. Toxicol. Occup. Med. 4: 281-287.

Koga, N.; Beppu, M.; Ishida, C.; Yoshimura, H. (1989) Further studies on metabolism in vivo of 3,4,3',4'-tetrachlorobiphenyl in rats: identification of minor metabolites in rat faeces. Xenobiotica 19(11): 1307-1318.

Kohn, M.C.; Lucier, G.W.; Clark, G.W.; Sewall, G.C.; Tritscher, A.M.; Portier, C.J. (1993) A mechanistic model of effects of dioxin on gene expression in the rat liver. Toxicol. Appl. Pharmacol. 120: 138-154.

Korfmacher, W.A.; Hansen, E.B., Jr.; Rowland, K.L. (1986) Tissue distribution of 2,3,7,8-TCDD in bullfrogs obtained from a 2,3,7,8-TCDD-contaminated area. Chemosphere 15(2): 121-126.

Korte, M.; Golor, G.; Stahlmann, R.; Chahoud, I.; Neubert, D. (1989) Elimination of TCDD from lactating rats in relation to the litter size. Teratology 40: 287.

Korte, M.; Stahlmann, R.; Neubert, D. (1990) Induction of hepatic monooxygenases in female rats and offspring in correlation with TCDD tissue concentrations after single treatment during pregnancy. Chemosphere 20(7-9): 1193-1198.

Krowke, R. (1986) Studies on distribution and embryotoxicity of different PCDD and PCDF in mice and marmosets. Chemosphere 15(9-12): 2011-2022.

DRAFT--DO NOT QUOTE OR CITE

Krowke, R.; Chahoud, I.; Baumann-Wilschke, I.; Neubert, D. (1989) Pharmacokinetics and biological activity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 2. Pharmacokinetics in rats using a loading-dose/maintenance-dose regime with high doses. Arch. Toxicol. 63: 356-360.

Krowke, R.; Abraham, K.; Wiesmuller, T.; Hagenmaier, H.; Neubert, D. (1990) Transfer of various PCDDs and PCDFs via placenta and mother's milk to marmoset offspring. Chemosphere 20(7-9): 1065-1070.

Kurl, R.N.; Loring, J.M.; Vilee, C.A. (1985) Control of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin binding protein(s) in the hamster kidney. Pharmacology 30: 245-254.

Kuroki, H.; Masuda, Y.; Yoshihara, S.; Yoshimura, H. (1980) Accumulation of polychlorinated dibenzofurans in the livers of monkeys and rats. Food Cosmet. Toxicol. 18: 387-392.

Kuroki, H.; Haraguchi, K.; Masuda, Y. (1990) Metabolism of polychlorinated dibenzofurans (PCDFs) in rats. Chemosphere 20(7-9): 1059-1064.

Lakshmanan, M.R.; Campbell, B.S.; Chirtel, S.J.; Ekarohita, N.; Ezekiel, M. (1986) Studies on the mechanism of absorption and distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. J. Pharmacol. Exp. Therap. 239(3): 673-677.

Leung, H-W.; Ku, R.H.; Paustenbach, D.J.; Andersen, M.E. (1988) A physiologically based pharmacokinetic model for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in C57BL/6J and DBA/2J mice. Toxicol. Lett. 42: 15-28.

Leung, H-W.; Paustenbach, D.J.; Murray, F.J.; Andersen, M.E. (1990a) A physiological pharmacokinetic description of the tissue distribution and enzyme-inducing properties of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. Toxicol. Appl. Pharmacol. 103: 399-410.

Leung, H-W.; Poland, A.P. Paustenbach, D.J.; Andersen, M.E. (1990b) Dose dependent pharmacokinetics of [125I]-2-iodo-3,7,8-trichlorodibenzo-*p*-dioxin in mice: analysis with a physiological modeling approach. Toxicol. Appl. Pharmacol. 103: 411-419.

Leung, H-W.; Wendling, J.M.; Orth, R.; Hileman, F.; Paustenbach, D.J. (1990c) Relative distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in human hepatic and adipose tissues. Toxicol. Lett. 50: 275-282.

Lucier, G.W.; Sonawane, B.R.; McDaniel, O.S.; Hook, G.E.R. (1975) Postnatal stimulation of hepatic microsomal enzymes following administration of TCDD to pregnant rats. Chem. Biol. Interact. 11: 15-26.

DRAFT--DO NOT QUOTE OR CITE

Lucier, G.W.; McDaniel, O.S.; Schiller, C.M.; Matthews, H.B. (1978) Structural requirements for the accumulation of chlorinated biphenyl metabolites in the fetal rat intestine. *Drug Metab. Dispos.* 6(1): 584-590.

Lucier, G.W.; Rumbaugh, R.C.; McCoy, Z.; Hass, R.; Harvan, D.; Albro, P. (1986) Ingestion of soil contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) alters hepatic enzyme activities in rats. *Fund. Appl. Toxicol.* 6: 364-371.

Lutz, R.J.; Dedrick, R.L.; Matthews, H.B.; Eling, T.E.; Anderson, M.W. (1977) A preliminary pharmacokinetic model for several chlorinated biphenyls in the rat. *Drug Metab. Dispos.* 5(4): 386-396.

Lutz, R.J.; Dedrick, R.L.; Tuey, D.; Sipes, I.G.; Anderson, M.W.; Matthews, H.B. (1984) Comparison of the pharmacokinetics of several polychlorinated biphenyls in mouse, rat, dog and monkey by means of a physiological pharmacokinetic model. *Drug Metab. Dispos.* 12(5): 527-535.

Manara, L.; Coccia, P.; Croci, T. (1982) Persistent tissue levels of TCDD in the mouse and their reduction as related to prevention of toxicity. *Drug Metab. Rev.* 13(3): 423-446.

Manara, L.; Coccia, P.; Croci, T. (1984) Prevention of TCDD toxicity in laboratory rodents by addition of charcoal or choleic acids to chow. *Food Chem. Toxicol.* 22(10): 815-818.

Marinovich, M.; Sirtori, C.R.; Galli, C.L.; Paoletti, R. (1983) The binding of 2,3,7,8-tetrachlorodibenzodioxin to plasma lipoproteins may delay toxicity in experimental hyperlipidemia. *Chem.-Biol. Interact.* 45: 393-399.

Mason, G.; Safe, S. (1986) Synthesis, biologic and toxic properties of 2,3,7,8-TCDD metabolites. *Chemosphere* 15(9-12): 2081-2083.

Mason, G.; Safe, S. (1986) Synthesis, biologic and toxic effects of the major 2,3,7,8-tetrachlorodibenzo-*p*-dioxin metabolites in the rat. *Toxicology* 41: 153-159.

Masuda, Y.; Kuroki, H.; Haraguchi, K.; Nagayama, J. (1985) PCB and PCDF congeners in the blood and tissues of Yusho and Yu-Cheng patients. *Environ. Health Perspect.* 59: 53-58.

Matthews, H.B.; Dedrick, R.L. (1984) Pharmacokinetics of PCBs. *Ann. Rev. Pharmacol. Toxicol.* 24: 85-103.

McConnell, E.E.; Lucier, G.W.; Rumbaugh, R.C.; et al. (1984) Dioxin in soil: bioavailability after ingestion by rats and guinea pigs. *Science* 223: 1077-1079.

DRAFT--DO NOT QUOTE OR CITE

McKinley, M.K.; Dedderis, L.B.; Birnbaum, L.S. (1993) The effect of pretreatment on the biliary excretion of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 2,3,7,8-tetrachlorodibenzofuran, and 3,3',4,4'-tetrachlorobiphenyl in the rat. *Fund. Appl. Toxicol.* 21: 425-432.

McKinney, J.D.; Chae, K.; Oatley, S.J.; Blake, C.C.F. (1985) Molecular interactions of toxic chlorinated dibenzo-*p*-dioxins and dibenzofurans with thyroxine binding prealbumin. *J. Med. Chem.* 28: 375-381.

McLachlan, M.S. (1993) Digestive tract absorption of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in a nursing infant. *Toxicol. Appl. Pharmacol.* 123: 68-72.

McNulty, W.P.; Nielsen-Smith, K.A.; Lay, J.O.; et al. (1982) Persistence of TCDD in monkey adipose tissue. *Food Chem. Toxic.* 20: 985-987.

Millis, C.D.; Mills, R.A.; Sleight, S.D.; Aust, S.D. (1985) Toxicity of 3,4,5,3',4',5'-hexabrominated biphenyl and 3,4,3',4'-tetrabrominated biphenyl. *Toxicol. Appl. Pharmacol.* 78: 88-95.

Mills, R.A.; Millis, C.D.; Dannan, G.A.; Guengerich, F.P.; Aust, S.D. (1985) Studies on the structure-activity relationships for the metabolism of polybrominated biphenyls by rat liver microsomes. *Toxicol. Appl. Pharmacol.* 78: 96-104.

Miyata, H.; Takayama, K.; Ogaki, J.; Mimura, M.; Kashimoto, T.; Yamada, T. (1989) Levels of PCDDs, coplanar PCBs and PCDFs in patients with Yusho disease and in the Yusho oil. *Chemosphere* 18(1-6): 407-416.

MMWR (Morbidity and Mortality Weekly Report). (1988) Serum 2,3,7,8-tetrachlorodibenzo-*p*-dioxin levels in Air Force health study participants—preliminary report. Atlanta, GA: Centers for Disease Control; 3(20): 309-311.

Moore, J.A.; Harris, M.W.; Albro, P.W. (1976) Tissue distribution of [¹⁴C] tetrachlorodibenzo-*p*-dioxin in pregnant and neonatal rats. *Toxicol. Appl. Pharmacol.* 37(1): 146-147.

Morita, M.; Oishi, S. (1977) Clearance and tissue distribution of polychlorinated dibenzofurans in mice. *Bull. Environ. Contam. Toxicol.* 18(1): 61-66.

NATO/CCMS (North Atlantic Treaty Organization, Committee on the Challenges of Modern Society). (1988) International toxicity equivalency factor (I-TEF) method of risk assessment for complex mixtures of dioxins and related compounds. Report No. 176.

Nau, H.; Bass, R. (1981) Transfer of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to the mouse embryo and fetus. *Toxicology* 20: 299-308.

Nau, H.; Bab, R.; Neubert, D. (1986) Transfer of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) via placenta and milk, and postnatal toxicity in the mouse. *Arch. Toxicol.* 59: 36-40.

Nauman, C.H.; Schaum, J.L. (1987) Human exposure estimation for 2,3,7,8-TCDD. *Chemosphere* 16(8/9): 1851-1856.

Neal, R.A.; Olson, J.R.; Gasiewicz, T.A.; Geiger, L.E. (1982) The toxicokinetics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in mammalian systems. *Drug Metab. Rev.* 13(3): 355-385.

Nelson, J.O.; Menzer, R.E.; Kearney, P.C.; Plimmer, J.R. (1977) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: in vitro binding to rat liver microsomes. *Bull. Environ. Contam. Toxicol.* 18(1): 9-13.

Nessel, C.S.; Amoroso, M.A.; Umbreit, T.H.; Gallo, M.A. (1990) Hepatic aryl hydrocarbon hydroxylase and cytochrome P450 induction following the transpulmonary absorption of TCDD from intratracheally instilled particles. *Fund. Appl. Toxicol.* 15: 500-509.

Nessel, C.S.; Amoroso, M.A.; Umbreit, T.H.; Meeker, R.J.; Gallo, M.A. (1992) Pulmonary bioavailability and fine particle enrichment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in respirable soil particles. *Fund. Appl. Toxicol.* 19: 279-285.

Neubert, D.; Wiesmuller, T.; Abraham, K.; Krowke, R.; Hagenmaier, H. (1990) Persistence of various polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs and PCDFs) in hepatic and adipose tissue of marmoset monkeys. *Arch. Toxicol.* 64: 431-442.

Neupert, M.; Weis, H.; Stock, B.; Thies, J.; Bayer, A.G. (1989) Analytical procedures in connection with acute toxicity studies. I. Tetrabromodibenzo-*p*-dioxin (TBDD). *Chemosphere* 19(1-6): 115-120.

Nolan, R.J.; Smith, F.A.; Hefner, J.G. (1979) Elimination and tissue distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in female guinea pigs following a single oral dose. *Toxicol. Appl. Pharmacol.* 48(1): A162.

Norback, D.H.; Engblom, J.F.; Allen, J.R. (1975) Tissue distribution and excretion of octachlorodibenzo-*p*-dioxin in the rat. *Toxicol. Appl. Pharmacol.* 32: 330-338.

DRAFT--DO NOT QUOTE OR CITE

Noren, K.; Lunden, A.; Sjobvall, J.; Bergman, A. (1990) Coplanar polychlorinated biphenyls in Swedish human milk. *Chemosphere* 20(7-9): 935-941.

Nygren, M.; Rappe, C.; Linstrom, G.; et al. (1986) Identification of 2,3,7,8-substituted polychlorinated dioxins and dibenzofurans in environmental and human samples. In: Rappe, C.; Chouhary, G.; Keith, L.H., eds. *Chlorinated dioxins and dibenzofurans in perspective*. Chelsea, MI: Lewis Publishers, Inc.; pp. 17-34.

Olafsson, P.G.; Bryan, A.M.; Stone, W. (1988) Polychlorinated biphenyls and polychlorinated dibenzofurans in the tissues of patients with Yusho or Yu-Chen. Total toxicity. *Bull. Environ. Contam. Toxicol.* 41: 63-70.

Olson, J.R. (1986) Metabolism and disposition of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in guinea pigs. *Toxicol. Appl. Pharmacol.* 85: 263-273.

Olson, J.R.; Gasiewicz, T.A.; Neal, R.A. (1980) Tissue distribution, excretion, and metabolism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the golden syrian hamster. *Toxicol. Appl. Pharmacol.* 56(1): 78-85.

Olson, J.R.; Gudzinowicz, M.; Neal, R.A. (1981) The in vitro and in vivo metabolism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the rat. *Toxicologist* 1: 69-70.

Olson, J.R.; Gasiewicz, T.A.; Geiger, L.E.; Neal, R.A. (1983) The metabolism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in mammalian systems. In: Coulston, R.; Pocchiari, F., eds. *Accidental exposure dioxins: human health aspects*. New York, NY: Academic Press; pp. 81-100.

Olson, J.R.; McGarrigle, B.P.; Gigliotti, P.J.; Kumar, S.; McReynolds, J.H. (1994) Hepatic uptake and metabolism of 2,3,7,8-TCDD and 2,3,7,8-TCDF. *Fund. Appl. Toxicol.* (in press).

Patterson, D.G.; Holler, J.S.; Lapeza, C.R., Jr.; et al. (1986) High-resolution gas chromatographic/high-resolution mass spectrometric analysis of human adipose tissue for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Anal. Chem.* 58: 705-713.

Patterson, D.G., Jr.; Hampton, L.; Lapeza, C.R., Jr.; et al. (1987) High-resolution gas chromatographic/high-resolution mass spectrometric analysis of human serum on a whole-weight and lipid basis for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Anal. Chem.* 59: 2000-2005.

Patterson, D.G., Jr.; Needham, L.L.; Pirkle, J.L.; et al. (1988) Correlation between serum and adipose tissue levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in 50 persons from Missouri. *Arch. Environ. Contam. Toxicol.* 17: 139-143.

DRAFT--DO NOT QUOTE OR CITE

Patterson, D.G., Jr.; Fingerhut, M.A.; Roberts, D.W.; et al. (1989a) Levels of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Am. J. Ind. Med.* 16: 135-146.

Patterson, D.G., Jr.; Fürst, P.; Henderson, L.O.; Ianance, S.G.; Alexander, L.R.; Turner, W.R.; Needham, L.L.; Hannon, H. (1989b) Partitioning of in vivo bound PCDDs/PCDFs among various compartments in whole blood. *Chemosphere* 19(1-6): 135-142.

Pedersen, L.G.; Darden, T.A.; Oatley, S.J.; McKinney, J.D. (1986) A theoretical study of the binding of polychlorinated biphenyls (PCBs) dibenzodioxins, and dibenzofuran to human plasma prealbumin. *J. Med. Chem.* 29: 2451-2457.

Philippi, M.; Krasnobagew, V.; Zeyer, J.; Huetter, R. (1981) Fate of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in microbial cultures and soil under laboratory conditions. *FEMS Symp.* 12: 2210-2233.

Phillips, D.L. (1989) Propagation of error and bias in half-life estimates based on two measurements. *Arch. Environ. Contam. Toxicol.* 18: 508-514.

Piper, W.N.; Rose, J.Q.; Gehring, P.J. (1973) Excretion and tissue distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. *Environ. Health. Perspec.* 5: 241-244.

Pirkle, J.L.; Wolfe, W.H.; Patterson, D.G.; et al. (1989) Estimates of the half-life of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in Vietnam veterans of operation Ranch Hand. *J. Toxicol. Environ. Health.* 27: 165-171.

Pluess, N.; Poiger, H.; Schlatter, C.; Buser, H.R. (1987) The metabolism of some pentachlorodibenzofurans in the rat. *Xenobiotica* 17(2): 209-216.

Pohjanvirta, R.; Vartiainen, T.; Uusi-Rauva, A.; Monkkonen, J.; Tuomisto, J. (1990) Tissue distribution, metabolism, and excretion of [¹⁴C]-TCDD in a TCDD-susceptible and a TCDD-resistant rat strain. *Pharmacol. Toxicol.* 66: 93-100.

Poiger, H.; Buser, H.R. (1984) The metabolism of TCDD in the dog and rat. In: Poland, A.; Kimbrough, R.D., eds. *Biological mechanisms of dioxin action*, vol. 18; Banbury Report. Cold Spring Harbor Laboratory; pp. 39-47.

Poiger, H.; Schlatter, C. (1979) Biological degradation of TCDD in rats. *Nature.* 281: 706-707.

Poiger, H.; Schlatter, C. (1980) Influence of solvents and adsorbents on dermal and intestinal absorption of TCDD. *Food Cosmet. Toxicol.* 18: 477-481.

DRAFT--DO NOT QUOTE OR CITE

Poiger, H.; Schlatter, C. (1985) Influence of phenobarbital and TCDD on the hepatic metabolism of TCDD in the dog. *Experientia* 41: 376-378.

Poiger, H.; Schlatter, C. (1986) Pharmacokinetics of 2,3,7,8-TCDD in man. *Chemosphere* 15(9-12): 1489-1494.

Poiger, H.; Buser, H.-R.; Weber, H.; Zweifel, U.; Schlatter, C. (1982) Structure elucidation of mammalian TCDD-metabolites. *Experientia* 38: 484-486.

Poiger, H.; Buser, H.R.; Schlatter, C. (1984) *Chemosphere* 13: 351-357.

Poiger, H.; Pluess, N.; Buser, H.R. (1989a) The metabolism of selected PCDFs in the rat. *Chemosphere* 18(1-6): 259-264.

Poiger, H.; Pluess, N.; Schlatter, C. (1989b) Subchronic toxicity of some chlorinated dibenzofurans in rats. *Chemosphere* 18(1-6): 265-275.

Poland, A.; Glover, E. (1970) An estimate of the maximum in vivo covalent binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to rat liver protein, ribosomal RNA, and DNA. *Cancer Res.* 39: 3341-3344.

Poland, A.; Teitelbaum, P.; Glover, E. (1989a) [¹²⁵I]2-iodo-3,7,8-trichlorodibenzo-*p*-dioxin-binding species in mouse liver induced by agonists for the Ah receptor: characterization and identification. *Molec. Pharmacol.* 36: 113-120.

Poland, A.; Teitelbaum, P.; Glover, E.; Kende, A. (1989b) Stimulation of in vivo hepatic uptake and in vitro hepatic binding of [¹²⁵I]2-iodo-3,7,8-trichlorodibenzo-*p*-dioxin by the administration of agonists for the Ah receptor. *Molec. Pharmacol.* 36: 121-127.

Rahman, M.S.; Zatz, J.L.; Umbreit, T.H.; Gallo, M.A. (1992) Comparative in vitro permeation of 2,3,7,8-TCDD through hairless mouse and human skin. *Toxicologist* 12: 80.

Ramsey, J.C.; Hefner, J.G.; Karbowski, R.J.; Braun, W.H.; Gehring, P.J. (1979) The in vivo biotransformation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the rat. *Toxicol. Appl. Pharmacol.* 48: A162.

Ramsey, J.C.; Hefner, J.G.; Karbowski, R.J.; Braun, W.H.; Gehring, P.J. (1982) The in vivo biotransformation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the rat. *Toxicol. Appl. Pharmacol.* 65: 180-184.

- Rappe, C. (1984) Analysis of polychlorinated dioxins and furans. All 75 PCDDs and 135 PCDFs can be identified by isomer-specific techniques. *Environ. Sci. Technol.* 18(3): 78A-90A.
- Rappe, C.; Nyhgren, M.; Marklund, S.; et al. (1985) Assessment of human exposure to polychlorinated dibenzofurans and dioxins. *Environ. Health Perspect.* 60: 303-304.
- Rappe, C.; Nygren, M.; Lindström, G.; Hansson, M. (1986) Dioxins and dibenzofurans in biological samples of European origin. *Chemosphere* 15: 1635-1639.
- Rappe, C.; Tarkowski, S.; Yrjanheikki, E. (1989) The WHO/EURO quality control study on PCDDs and PCDFs in human milk. *Chemosphere* 18(1-6): 883-889.
- Rau, L.A.; Vodcnik, M.J. (1986) Mechanisms for the release and redistribution of 2,4,5,2',4',5'-hexachlorobiphenyl (6-CB) from hepatic tissues in the rat. *Fund. Appl. Toxicol.* 7: 494-501.
- Reggiani, G. (1980) Acute human exposure to TCDD in Sevesco, Italy. *J. Toxicol. Environ. Health.* 6(1): 27-43.
- Ring, B.J.; Seitz, K.R.; Vodcnik, M.J. (1988) Transfer of 2,4,5,2',4',5'-hexachlorobiphenyl across the in situ perfused guinea pig placenta. *Toxicol. Appl. Pharmacol.* 96: 7-13.
- Ring, B.J.; Seitz, K.R.; Gallenberg, L.A.; Vodcnik, M.J. (1990) The effect of diet and litter size on the elimination of 2,4,5,2',4',5'-[¹⁴C]hexachlorobiphenyl from lactating mice. *Toxicol. Appl. Pharmacol.* 104: 9-16.
- Rose, J.Q.; Ramsey, J.C.; Wentzler, T.H.; Hummel, R.A.; Gehring, P.J. (1976) The fate of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin following single and repeated oral doses to the rat. *Toxicol. Appl. Pharmacol.* 36: 209-226.
- Rozman, K. (1984) Hexadecane increases the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD): is brown adipose tissue the primary target in TCDD-induced wasting syndrome? *Biochem. Biophys. Res. Commun.* 125(3): 996-1004.
- Ryan, J.J. (1986) Variation of dioxins and furans in human tissues. *Chemosphere* 15: 1585-1593.

Ryan, J.J.; Masuda, Y. (1989) Half-lives for elimination of polychlorinated dibenzofurans (PCDFs) and PCBs in humans from the Yusho and Yucheng rice oil poisonings. In: Proceedings of 9th international symposium on chlorinated dioxins and related compounds, Dioxin '89; September 17-22, 1989; Toronto, Ont.; p. TOX06.

Ryan, J.J.; Masuda, Y. (1991) Elimination of polychlorinated dibenzofurans (PCDFs) in humans from the Yusho and Yucheng rice oil poisonings. In: Proceedings of 11th international symposium on chlorinated dioxins and related compounds, Dioxin '91; September 23-27, 1991; Research Triangle Park, NC; p. 70.

Ryan, D.E.; Thomas, P.E.; Levin, W. (1980) Hepatic microsomal cytochrome P-450 from rats treated with isosafrole. J. Biol. Chem. 255: 7941-7955.

Ryan, J.J.; Schecter, A.; Lizotte, R.; Sun, W-F.; Miller, L. (1985a) Tissue distribution of dioxins and furans in humans from the general population. Chemosphere 14(6/7): 929-932.

Ryan, J.J.; Lizotte, R.; Lau, B.P.Y. (1985b) Chlorinated dibenzo-*p*-dioxins and chlorinated dibenzofurans in Canadian human adipose tissue. 14(6-7): 697-706.

Ryan, J.J.; Lizotte, R.; Lewis, D. (1987) Human tissue levels of PCDDs and PCDFs from a fatal pentachlorophenol poisoning. Chemosphere 16(8/9): 1989-1996.

Ryan, J.J.; Gasiewicz, T.A.; Brown, J.F., Jr. (1990) Human body burden of polychlorinated dibenzofurans associated with toxicity based on the Yusho and Yucheng incidents. Fund. Appl. Toxicol. 15: 722-731.

Sawahata, T.; Olson, J.R.; Neal, R.A. (1982) Identification of metabolites of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) formed on incubation with isolated rat hepatocytes. Biochem. Biophys. Res. Commun. 105(1): 341-346.

Schecter, A. (1991) Dioxins and related chemicals in humans and the environment. In: Banbury Report 35. Biological basis for risk assessment of dioxins and related compounds. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; pp. 169-212.

Schecter, A.; Ryan, J.J. (1989) Blood and adipose tissue levels of PCDDs/ PCDFs over three years in a patient after exposure to polychlorinated dioxins and dibenzofurans. Chemosphere 18(1-6): 635-642.

Schecter, A.; Tiernan, T.; Schaffner, F.; et al. (1985) Patient fat biopsies for chemical analysis and liver biopsies for ultrastructural characterization after exposure to polychlorinated dioxins, furans and PCBs. Environ. Health Perspec. 60: 241-254.

DRAFT--DO NOT QUOTE OR CITE

Schechter, A.J.; Ryan, J.J.; Constable, J.D. (1986) Chlorinated dibenzo-*p*-dioxin and dibenzofuran levels in human adipose tissue and milk samples from the north and south of Vietnam. *Chemosphere* 15: 1613-1620.

Schechter, A.J.; Ryan, J.J.; Constable, J.D. (1987) Polychlorinated dibenzo-*p*-dioxin and polychlorinated dibenzofuran levels in human breast milk from Vietnam compared with cow's milk and human breast milk from the North American continent. *Chemosphere* 16(8-9): 2003-2016.

Schechter, A.; Ryan, J.J.; Constable, J.D. (1989) Chlorinated dioxins and dibenzofurans in human milk from Japan, India, and the United States of America. *Chemosphere* 18(1-6): 975-980.

Schechter, A.; Ryan, J.J.; Kostyniak, P.J. (1990a) Decrease over a six year period of dioxin and dibenzofuran tissue levels in a single patient following exposure. *Chemosphere* 20(7-9): 911-917.

Schechter, A.; Ryan, J.J.; Constable, J.D.; Baughman, R.; Banger, J.; Furst, P.; Wilmers, K.; Oates, R.D. (1990b) Partitioning of 2,3,7,8-chlorinated dibenzo-*p*-dioxins and dibenzofurans between adipose tissue and plasma lipid of 20 Massachusetts Vietnam veterans. *Chemosphere* 20: 951-958.

Scheuplein, R.J.; Shoaf, S.E.; Brown, R.N. (1990) Role of pharmacokinetics in safety evaluation and regulatory considerations. *Ann. Rev. Pharmacol. Toxicol.* 30: 197-218.

Schnellmann, R.G.; Vickers, A.E.M.; Sipes, I.G. (1985) Metabolism and disposition of polychlorinated biphenyls. In: Hodgson, E.; Bend, J.R.; Philpot, R.M.; eds. *Reviews in Biochemical Toxicology*, vol. 7. Amsterdam: Elsevier Press; p. 247-282.

Sewall, C.; Lucier, G.; Tritscher, A.; Clark, G. (1992) Dose-response for TCDD-mediated changes in hepatic EGF receptor in an initiation-promotion model for hepatocarcinogenesis in female rats. *Cancer Res.* 52: 3436-3442.

Shen, E.S.; Olson, J.R. (1987) Relationship between the murine Ah phenotype and the hepatic uptake and metabolism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Drug Metab. Dispos.* 15(5): 653-660.

Shimada, T. (1987) Lack of correlation between formation of reactive metabolites and thymic atrophy caused by 3,4,3',4'-tetrachlorobiphenyl in C57BL/6N mice. *Arch. Toxicol.* 59: 301-306.

- Shimada, T.; Sawabe, Y. (1983) Activation of 3,4,3',4'-tetrachlorobiphenyl to protein-bound metabolites by rat liver microsomal cytochrome P-448-containing monooxygenase system. *Toxicol. Appl. Pharmacol.* 70: 486-493.
- Shireman, R.B.; Wei, C. (1986) Uptake of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin from plasma lipoproteins by cultured human fibroblasts. *Chem.-Biol. Interact.* 58: 1-12.
- Shu, H.; Paustenbach, D.; Murray, F.J.; et al. (1988a) Bioavailability of soil-bound TCDD: oral bioavailability in the rat. *Fund. Appl. Toxicol.* 10: 648-654.
- Shu, H.; Teitelbaum, P.; Webb, A.S.; et al. (1988b) Bioavailability of soil-bound TCDD: dermal bioavailability in the rat. *Fund. Appl. Toxicol.* 10: 335-343.
- Sielken, R.L., Jr. (1987) Statistical evaluations reflecting the skewness in the distribution of TCDD levels in human adipose tissue. *Chemosphere* 16(8/9): 2135-2140.
- Sijm, D.T.H.M.; Wever, H.; Opperhuizen, A. (1989) Influence of biotransformation on the accumulation of PCDDs from fly-ash in fish. *Chemosphere* 19(1-6): 475-480.
- Sijm, D.T.H.M.; Yarechewski, A.L.; Muir, D.C.G.; Barrie Webster, G.R.; Seinen, W.; Opperhuizen, A. (1990) Biotransformation and tissue distribution of 1,2,3,7-tetrachlorodibenzo-*p*-dioxin, 1,2,3,4,7-pentachlorodibenzo-*p*-dioxin and 2,3,4,7,8-pentachlorodibenzofuran in rainbow trout. *Chemosphere* 21(7): 845-866.
- Sipes, I.G.; Slocumb, M.L.; Perry, D.F.; Carter, D.E. (1982) 2,4,5,2',4',5'-hexachlorobiphenyl: distribution, metabolism, and excretion in the dog and the monkey. *Toxicol. Appl. Pharmacol.* 65: 264-272.
- Souès, S.; Fernandez, N.; Souverain, P.; Lesca, P. (1989a) Separation of the different classes of intrahepatic lipoproteins from various animal species. Their binding with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and benzo(a)pyrene. *Biochem. Pharmacol.* 38(17): 2833-2839.
- Souès, S.; Fernandez, N.; Souverain, P.; Lesca, P. (1989b) Intracellular lipoproteins as carriers for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and benzo(a)pyrene in rat and mouse liver. *Biochem. Pharmacol.* 38(17): 2841-2847.
- Stanley, J.S.; Ayling, R.E.; Cramer, P.H.; et al. (year) Polychlorinated dibenzo-*p*-dioxin and dibenzofuran concentration levels in human adipose tissue samples from the continental United States collected from 1971 through 1987. *Chemosphere* 20(7-9): 895-901.

DRAFT--DO NOT QUOTE OR CITE

Tai, H.; McReynolds, J.H.; Goldstein, J.A.; Eugster, H.; Sengstag, C.; Alworth, W.L.; Olson, J.R. (1993) Cytochrome P5401A1 mediates the metabolism of 2,3,7,8-tetrachlorodibenzofuran in the rat and human. *Toxicol. Appl. Pharmacol.* 123:34-42.

Thoma, H.; Mucke, W.; Kretschmer, E. (1989) Concentrations of PCDD and PCDF in human fat and liver samples. *Chemosphere* 18(1-6): 491-498.

Thoma, H.; Mücke, W.; Kauert, G. (1990) Comparison of the polychlorinated dibenzo-*p*-dioxin and dibenzofuran in human tissue and human liver. *Chemosphere* 20(3/4): 433-442.

Tong, H.Y.; Gross, M.L.; Bowman, R.E.; Monson, S.J.; Weerasinghe, N.C.A. (1989) Controlled exposure of female rhesus monkeys to 2,3,7,8-TCDD: concentrations of TCDD in fat of mothers and offspring. In: *Proceedings of 9th international symposium on chlorinated dioxins and related compounds, Dioxin '89; September 17-22, 1989; Toronto, Ont.;* p. TOX2.

Travis, C.C.; Hattemer-Frey, H.A. (1987) Human exposure to 2,3,7,8-TCDD. *Chemosphere* 16(10/12): 2331-2342.

Tritscher, A.M.; Goldstein, J.A.; Portier, C.J.; McCoy, Z.; Clark, G.C.; Lucier, G.W. (1992) Dose-response relationships for chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a rat tumor promotion model: quantification and immunolocalization of CYP1A1 and CYP1A2 in the liver. *Cancer Res.* (in press).

Tuey, D.B.; Matthews, H.B. (1980) Distribution and excretion of 2,2',4,4',5,5'-hexabromobiphenyl in rats and man: pharmacokinetic model predictions. *Toxicol. Appl. Pharmacol.* 53: 420-431.

Tuteja, N.; Gonzalez, F.J.; Nebert, D.W. (1985) Developmental and tissue differential regulation of the mouse dioxin-inducible P1-450 and P3-450 genes. *Dev. Biol.* 112: 177-184.

Umbreit, T.H.; Hesse, E.J.; Gallo, M.A. (1986a) Bioavailability of dioxin in soil from a 2,4,5,-T manufacturing site. *Science.* 232: 497-499.

Umbreit, T.H.; Hesse, E.J.; Gallo, M.A. (1986b) Comparative toxicity of TCDD contaminated soil from Times Beach, Missouri, and Newark, New Jersey. *Chemosphere* 15(9-12): 2121-2124.

U.S. EPA. (1989) Interim procedures for estimating risks associated with exposures to mixtures of chlorinated dibenzo-*p*-dioxins and dibenzofurans (CDDs and CDFs) and 1989 update. Washington, DC: Risk Assessment Forum.

Van den Berg, M.; Poiger, H. (1989) Selective retention of PCDDs and PCDFs in mammals: a multiple cause problem. *Chemosphere* 18(1-6): 677-680.

Van den Berg, M.; Sinke, M.; Wever, H. (1987a) Vehicle dependent bioavailability of polychlorinated dibenzo-*p*-dioxins (PCDDs) and -dibenzofurans (PCDFs) in the rat. *Chemosphere* 16(6): 1193-1203.

Van den Berg, M.; Heeremans, C.; Veenhoven, E.; Olie, K. (1987b) Transfer of polychlorinated dibenzo-*p*-dioxins and dibenzofurans to fetal and neonatal rats. *Fund. Appl. Toxicol.* 9: 635-644.

Van den Berg, M.; Bouwman, C.; Seinen, W. (1989a) Hepatic retention of PCDDs and PCDFs in C57B1/6 and DBA/2 mice. *Chemosphere* 19(1-6): 795-802.

Van den Berg, M.; de Jongh, J.; Eckhart, P.; Van der Wielen, F.W.M. (1989b) Disposition and elimination of three polychlorinated dibenzofurans in the liver of the rat. *Fund. Appl. Toxicol.* 12: 738-747.

Van den Berg, M.; de Jongh, J.; Eckhart, P.; Van der Wielen, F.W.M. (1989c) The elimination and absence of pharmacokinetic interaction of some polychlorinated dibenzofurans (PCDFs) in the liver of the rat. *Chemosphere* 18(1-6): 665-675.

Van den Berg, M.; van Wijnen, J.; Wever, H.; Seinen, W. (1989d) Selective retention of toxic polychlorinated dibenzo-*p*-dioxins and dibenzofurans in the liver of the rat after intravenous administration of a mixture. *Toxicology* 55: 173-182.

Van den Berg, M.; de Jongh, J.; Poiger, H.; Olson, J.R. (1994) The toxicokinetics and metabolism of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and their relevance for toxicity.

Van Miller, J.P.; Marlar, R.J.; Allen, J.R. (1976) Tissue distribution and excretion of tritiated tetrachlorodibenzo-*p*-dioxin in non-human primates and rats. *Food Cosmet. Toxicol.* 14: 31-34.

Veerkamp, W.; Serne, P.; Hutzinger, O. (1983) Prediction of hydroxylated metabolites in polychlorodibenzo-*p*-dioxins and polychlorodibenzofurans by Huckel molecular orbital calculations. *Chem. Soc. Perkin Trans. II*: 353-358.

Vinopal, J.H.; Casida, J.E. (1973) Metabolic stability of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in mammalian liver microsomal systems and in living mice. *Arch. Environ. Contam. Toxicol.* 1(2): 122-132.

DRAFT--DO NOT QUOTE OR CITE

Vodicnik, M.J.; Lech, J.J. (1980) The transfer of 2,4,5,2',4',5'-hexachlorobiphenyl to fetuses and nursing offspring. I. Disposition in pregnant and lactating mice and accumulation in young. *Toxicol. Appl. Pharmacol.* 54: 293-300.

Vodicnik, M.J.; Elcombe, C.R.; Lech, J.J. (1980) The transfer of 2,4,5,2',4',5'-hexachlorobiphenyl to fetuses and nursing offspring. II. Induction of hepatic microsomal monooxygenase activity in pregnant and lactating mice and their young. *Toxicol. Appl. Pharmacol.* 54: 301-310.

Voorman, R.; Aust, S.D. (1987) Specific binding of polyhalogenated aromatic hydrocarbon inducers of cytochrome P-450d to the cytochrome and inhibition of its estradiol 2-hydroxylase activity. *Toxicol. Appl. Pharmacol.* 90: 69-78.

Voorman, R.; Aust, S.D. (1989) TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) is a tight binding inhibitor of cytochrome P-450d. *J. Biochem. Toxicol.* 4: 105-109.

Wacker, R.; Poiger, H.; Schlatter, C. (1986) Pharmacokinetics and metabolism of 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin in the rat. *Chemosphere* 15(9-12): 1473-1476.

Weber, H.; Birnbaum, L.S. (1985) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) in pregnant C57BL/6N mice: distribution to the embryo and excretion. *Arch. Toxicol.* 57: 157-162.

Weber, H.; Poiger, H.; Schlatter, C. (1982a) Acute oral toxicity of TCDD-metabolites in male guinea pigs. *Toxicol. Lett.* 14: 117-122.

Weber, H.; Poiger, H.; Schlatter, C. (1982b) Fate of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Xenobiotica* 12(6): 353-357.

Weber, L.W.D.; Zesch, A.; Rozman, K. (1991) Penetration, distribution and kinetics of 2,3,7,8-TCDD in human skin in vitro. *Arch. Toxicol.* 65: 421-428.

Wehler, E.K.; Bergman, A.; Brandt, I.; Darnerud, P.O.; Wachtmeister, C.A. (1989) 3,3',4,4'-Tetrachlorobiphenyl excretion and tissue retention of hydroxylated metabolites in the mouse. *Drug Metabol. Dispos.* 17(4): 441-448.

Wehler, E.K.; Brunstrom, B.; Rannug, U.; Bergman, A. (1990) 3,3',4,4'-Tetrachlorobiphenyl: metabolism by the chick embryo in ovo and toxicity of hydroxylated metabolites. *Chem.-Biol. Interact.* 73: 121-132.

Weisiger, R.; Gollan, J.; Ockner, R. (1981) Receptor for albumin on the liver cell surface may mediate uptake of fatty acids and other albumin-bound substances. *Science* 211: 1048-1050.

Wendling, J.M.; Orth, R.G. (1990) Determination of [³H]-2,3,7,8-tetrachlorodibenzo-*p*-dioxin in human feces to ascertain its relative metabolism in man. *Anal. Chem.* 62: 796-800.

Wendling, J.; Hileman, F.; Orth, R.; Umbreit, T.; Hesse, E.; Gallo, J. (1989) An analytical assessment of the bioavailability of dioxin contaminated soils to animals. *Chemosphere* 18(1-6): 925-932.

Wendling, J.M.; Orth, R.G.; Poigner, H. (1990) Determination of [³H]-2,3,7,8-tetrachlorodibenzo-*p*-dioxin in human feces to ascertain its relative metabolism in man. *Anal. Chem.* 62(8): 796-800.

Wester, R.C.; Maibach, H.I.; Sedik, L.; Melendres, J.; Wade, M. (1993a) Percutaneous absorption of PCBs from soil: in vivo rhesus monkey, in vitro human skin, and binding to powdered human stratum corneum. *J. Toxicol. Environ Health.* 39(3): 375-382.

Wester, R.C.; Maibach, H.I.; Sedik, L.; Melendres, J.; Wade, M.; DiZio, S. (1993b) Percutaneous absorption of pentachlorophenol from soil. *Fund. Appl. Toxicol.* 20(1): 68-71.

Wiesmuller, T.; Bittmann, H.; Hagenmaier H.; et al. (1989) Uptake and distribution of 2,3,7,8-substituted PCDD and activities of key enzymes of the carbohydrate metabolism in mice dosed with different PCDD mixtures. In: *Proceedings of 9th international symposium on chlorinated dioxins and related compounds, Dioxin '89*; September 17-22, 1989; Toronto, Ont.; p. RTP11.

Williams, D.T.; Cunningham, H.M.; Blanchfield, B.J. (1972) Distribution and excretion studies of octachlorodibenzo-*p*-dioxin in the rat. *Bull. Environ. Contam. Toxicol.* 7(1): 57.

Wolff, M.S.; Thornton, J.; Fischbein, A.; Lilis, R.; Selikoff, I.J. (1982) Disposition of polychlorinated biphenyl congeners in occupationally exposed persons. *Toxicol. Appl. Pharmacol.* 62: 294-306.

Wroblewski, V.J.; Olson, J.R. (1985) Hepatic metabolism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the rat and guinea pig. *Toxicol. Appl. Pharmacol.* 81: 231-240.

Wroblewski, V.J.; Olson, J.R. (1988) Effect of monooxygenase inducers and inhibitors on the hepatic metabolism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat and hamster. *Drug Metab. Dispos.* 16(1): 43-51.

Wyss, P.A.; Muhlebach, S.; Bickel, M.H. (1982) Pharmacokinetics of 2,2',4,4',5,5'-hexachlorobiphenyl (6-CB) in rats with decreasing adipose tissue mass. I. Effects of restricting food intake two weeks after administration of 6-CB. *Drug Metab. Dispos.* 10(6): 657.

Wyss, P.A.; Muhlebach, S.; Bickel, M.H. (1986) Long-term pharmacokinetics of 2,2',4,4',5,5'-hexachlorobiphenyl (6-CB) in rats with constant adipose tissue mass. *Drug Metab. Dispos.* 14(3): 361

Yoshimura, H.; Kamimura, H.; Oguri, K.; Honda, Y.; Nakano, M. (1986) Stimulating effect of activated charcoal beads on fecal excretion of 2,3,4,7,8-pentachlorodibenzofuran in rats. *Chemosphere* 15(3): 219-227.

Yoshimura, H.; Kuroki, J.; Koga, N. (1987a) Unique features of subcellular distribution of 2,3,4,7,8-pentachlorodibenzofuran in rat liver. *Chemosphere* 16(8/9): 1695-1700.

Yoshimura, H.; Yonemoto, Y.; Yamada, H.; Koga, N.; Oguri, K.; Saeki, S. (1987b) Metabolism in vivo of 3,4,3',4',-tetrachlorobiphenyl and toxicological assessment of the metabolites in rats. *Xenobiotica* 17(8): 897-910.

Chapter 2. Mechanisms of Action

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

NOTICE

THIS DOCUMENT IS A PRELIMINARY DRAFT. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

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

DRAFT--DO NOT QUOTE OR CITE

DISCLAIMER

This document is an internal draft for review purposes only and does not constitute Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

CHAPTER 2. MECHANISM(S) OF ACTION

CONTENTS

2.1. INTRODUCTION	2-1
2.2. THE "RECEPTOR" CONCEPT	2-3
2.3. THE Ah (DIOXIN) RECEPTOR	2-6
2.4. THE Aroclor PROTEIN	2-9
2.5. OTHER PROTEINS THAT PARTICIPATE IN THE RESPONSE TO DIOXIN	2-11
2.6. ACTIVATION OF GENE TRANSCRIPTION BY DIOXIN	2-13
2.6.1. In Vitro Studies	2-13
2.6.2. In Vivo Studies	2-18
2.7. FUTURE RESEARCH	2-20
2.8. MECHANISTIC INFORMATION AND RISK ASSESSMENT	2-23
REFERENCES FOR CHAPTER 2	2-26

2. MECHANISM(S) OF ACTION*

2.1. INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) is the prototype for a class of halogenated aromatic hydrocarbons that produce similar patterns of toxicity and appear to have a common mechanism of action although they differ in potency (Poland and Knutson, 1982; Safe, 1986). Because it is the most potent, TCDD has been studied much more extensively than other structurally related compounds (Figure 2-1). TCDD achieved notoriety in the 1970s, when it was discovered to be a contaminant in the herbicide Agent Orange and was shown to produce birth defects in rodents. Subsequently, dioxin has continued to generate concern because of its widespread distribution, its persistence as an environmental contaminant, its accumulation within the food chain, and its toxic potency. In animals, TCDD elicits a wide range of biological effects, including alterations in metabolic pathways, immunological changes, teratogenic effects, and neoplasia (Poland and Knutson, 1982; Safe, 1986). In humans, the dioxin can produce the skin condition known as chloracne; the possibility that it also produces cancer and birth defects is a particular public health concern. Many individuals have been exposed to TCDD, primarily from dietary sources, although occupational and accidental exposures have also occurred. TCDD is a poor substrate for detoxification systems, such as the microsomal cytochrome P450 enzymes, which oxygenate other lipophilic compounds during their metabolic processing to inactive derivatives. Because of its relative resistance to metabolism, TCDD tends to persist in the body, and its half-life in humans is of the order of 7 to 10 years (Pirkle et al., 1989). Therefore, dioxin tends to accumulate in human tissues over time, raising the concern that repeated exposures, even to "low" concentrations, may evoke adverse health effects. Epidemiological studies have not produced a well-defined estimate of the health risk that dioxin poses to humans (Bailar, 1991), and there has been hope that knowledge of the

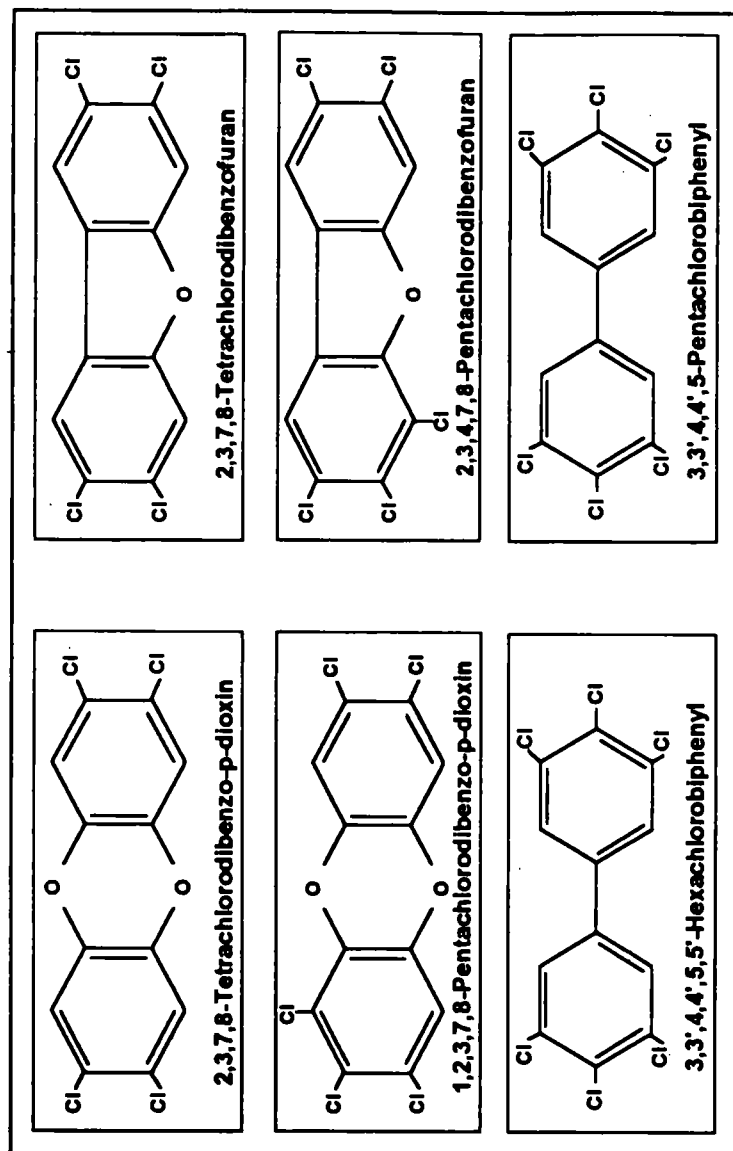


Figure 2-1. ICDD and related halogenated aromatic compounds.

mechanism of dioxin action may shed additional light on this issue (Gallo et al., 1991). Mechanistic studies can reveal the biochemical pathways and types of biological events that contribute to dioxin's adverse effects. For example, TCDD acts via an intracellular protein (the Ah receptor), which is a ligand-dependent transcription factor that functions in partnership with a second protein (known as Arnt); therefore, from a mechanistic standpoint, TCDD's adverse effects appear likely to reflect sustained alterations in gene expression. Mechanistic studies also indicate that several proteins contribute to TCDD's gene regulatory effects and that the response to TCDD probably involves a relatively complex interplay between multiple genetic and environmental factors. Such mechanistic information imposes constraints on the possible models that can plausibly account for TCDD's biological effects and, therefore, on the assumptions used during the risk assessment process. Mechanistic knowledge of dioxin action may also be useful in other ways. For example, knowledge of genetic polymorphisms that influence TCDD responsiveness may allow the identification of individuals at particular risk from exposure to dioxin. In addition, mechanistic knowledge of the biochemical pathways that are altered by TCDD may identify novel targets for the development of drugs that can antagonize dioxin's adverse effects.

As described below, biochemical and genetic analyses of the mechanism by which dioxin induces CYP1A1 gene transcription have revealed the outline of a novel regulatory system whereby a chemical signal can alter the expression of specific mammalian genes. Future studies of dioxin action have potential to provide additional new insights into mechanisms of mammalian gene regulation that are of relatively broad interest. Additional perspectives on dioxin action can be found in several recent reviews (Nebert et al., 1991; Silbergeld and Gasiewicz, 1989; Skene et al., 1989; Couture et al., 1990; Landers and Bunce, 1991; Johnson, 1992; Poellinger et al., 1992; Birnbaum, 1993).

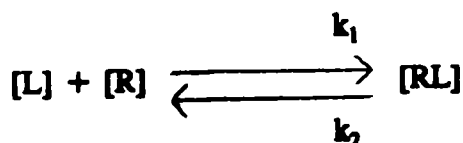
2.2. THE "RECEPTOR" CONCEPT

The idea that a drug, hormone, neurotransmitter, or other chemical produces a physiological response by interacting with a specific cellular target molecule, i.e., a "receptor," evolved from several observations. First, many chemicals elicit responses that are restricted to specific tissues. This type of observation implied that the responsive tissue

(i.e., the adrenal cortex) contained a "receptive" component whose presence was required for the physiologic effect (i.e., cortisol secretion). Second, many chemicals are quite potent. For example, nanomolar concentrations of numerous hormones and growth factors elicit biological effects. This type of observation suggested that the target cell contained a site(s) to which the chemical could bind with high affinity. Third, stereoisomers of some chemicals (i.e., catecholamines, opioids) differ by orders of magnitude in potency. This type of observation indicated that the molecular shape of the chemical strongly influenced its biological activity; this, in turn, implied that the binding site on or in the target cell also had a specific, three-dimensional configuration. Together, these types of observations predicted that the biological responses to some chemicals involve stereospecific, high-affinity binding of the chemical to specific receptor sites located on or in the target cell.

The availability of compounds of high specific radioactivity permitted quantitative analyses of their binding to tissue components in vitro. To qualify as a potential "receptor," a binding site for a given chemical must satisfy several criteria: (1) the binding site must be saturable (i.e., there should be a limited number of binding sites per cell); (2) the binding should be reversible; (3) the binding affinity measured in vitro should be consistent with the potency of the chemical observed in vivo; (4) if the biological response exhibits stereospecificity, so should the in vitro binding, (5) for a series of structurally related chemicals, the rank order for binding affinity should correlate with the rank order for biological potency; and (6) tissues that respond to the chemical should contain binding sites with the appropriate properties.

The binding of a chemical ("ligand") to its cognate receptor is assumed to obey the law of mass action, that is, it is a bimolecular, reversible interaction. The concentration of the liganded, or occupied, receptor [RL] is a function of both the ligand concentration [L] and the receptor concentration [R]:



[RL]

It follows that the fractional occupancy of the receptor $\frac{[RL]}{[R_{\text{tot}}]}$ is a function of the ligand concentration $[L]$ and a constant K_D , which is a measure of the binding affinity of the ligand for the receptor:

$$\frac{[RL]}{[R_{\text{tot}}]} = \frac{[L]}{K_D + [L]}$$

Therefore, the relationship between receptor occupancy and ligand concentration is hyperbolic. At low ligand concentrations (where $[L] \ll K_D$), a small increase in $[L]$ produces an approximately linear increase in fractional receptor occupancy. At high ligand concentration (where $[L] \gg K_D$), the fractional occupancy of the receptor is already very close to 1, and a small increase in $[L]$ produces no further meaningful increase in receptor occupancy, because the binding sites are essentially saturated.

Ligand binding constitutes only one aspect of the receptor concept. By definition, a receptor mediates a response, and the functional consequences of the ligand-receptor binding represent an essential aspect of the receptor concept. Receptor theory attempts to quantitatively relate ligand binding to biological response. The classical "occupancy" model of Clark (1933) postulated (1) that the magnitude of the biological response is directly proportional to the fraction of the occupied receptors, and (2) that the response is maximal when all receptors are occupied. However, analyses of numerous receptor-mediated effects indicate that the relationship between receptor occupancy and biological effect is not as straightforward as Clark envisioned. In certain cases, no response occurs even when there is some receptor occupancy, a threshold phenomenon that reflects the biological "inertia" of the response (Ariens et al., 1960). In other cases, a maximal response occurs well before all receptors are occupied, a phenomenon that reflects receptor "reserve" (Stephenson, 1956). Therefore, one cannot simply assume that the relationship between fractional receptor occupancy and biological response is linear. Furthermore, for a ligand (such as TCDD) that elicits multiple receptor-mediated effects, one cannot assume that the binding-response relationship for one, simple effect (such as enzyme induction) will necessarily be identical to the binding-response relationship for a different, more complex effect (such as cancer).

2.3. THE Ah (DIOXIN) RECEPTOR

The unusual potency of TCDD in eliciting its toxic effects suggested the possible existence of a receptor for the dioxin. Poland and coworkers, using radiolabeled TCDD as a ligand, demonstrated that the cytosolic fraction of C57BL/6 mouse liver contained a protein that bound the dioxin saturably (i.e., ~ 105 binding sites per cell), reversibly, and with high affinity (i.e., in the nanomolar range, consistent with TCDD's biological potency in vivo). Competition binding studies with congeners of TCDD revealed that ligands with the highest binding affinity were planar and contained halogen atoms in at least three of the four lateral positions; thus, ligand binding exhibited stereospecificity. In addition, the pattern of ligand binding in vitro resembled a rectangular hyperbola and, therefore, appeared to obey the law of mass action. Together, these findings indicated that the intracellular TCDD-binding protein had the ligand-binding properties expected for a "dioxin receptor." The protein is designated as the "Ah receptor" because it binds and mediates the response to other aromatic hydrocarbons (such as 3-methylcholanthrene) in addition to TCDD (Poland and Knutson, 1982).

The Ah receptor evolved prior to the introduction of halogenated aromatic hydrocarbons into the environment (Czuczwa et al., 1984). Therefore, some other compound(s) must represent the "natural" ligand(s) for the receptor (Poellinger et al., 1992). Naturally occurring high-affinity ligands for the receptor exist in the environment, particularly in plants (Gillner et al., 1985, 1989; Rannung et al., 1987; Bjeldanes et al., 1991). Thus, the Ah receptor might have evolved as part of a substrate-inducible system designed to metabolize dietary lipophilic substances, and TCDD may mimic the binding of such substances to the receptor. In some tissues, TCDD produces changes in the proliferative/differentiated phenotype of cells (Knutson and Poland, 1980). Thus, an additional possibility is that TCDD mimics an endogenous Ah receptor ligand that regulates such tissue-specific phenotypes.

Inbred mouse strains differ quantitatively in their responsiveness to TCDD and other aromatic hydrocarbons. For example, TCDD elicits its effects at about tenfold lower concentrations in the more responsive mouse strains (typified by C57BL/6) than in the less responsive strains (typified by DBA/2). The polymorphism is genetic in origin, and in

crossbreeding studies, the more responsive phenotype segregates as an autosomal dominant trait. Numerous responses to TCDD (e.g., enzyme induction, thymic involution, cleft palate formation, hepatic porphyria) exhibit a segregation pattern identical to that for the binding of TCDD to the Ah receptor. Thus, the genetic locus (designed Ah) that governs the receptor polymorphism also governs the biological responses to TCDD. These genetic findings implicate the Ah receptor in the mechanism of dioxin action (Poland and Knutson, 1982; Nebert et al., 1991).

The Ah receptor has been difficult to purify in substantial quantity, and insufficient knowledge of its structural and functional properties has represented a major impasse in our understanding of dioxin action. Biochemical studies of crude or partially purified receptor preparations indicate that the receptor is a soluble (as opposed to membrane-bound) intracellular protein, which, upon binding TCDD, acquires a high affinity for DNA. Studies of structure-activity relationships (SAR) reveal that, within groups of structurally related compounds, a ligand's receptor-binding affinity correlates with its potency in eliciting a biological response(s). Such SAR studies constitute biochemical evidence that implicates the Ah receptor in the mechanism of dioxin action. SAR analyses are useful for assessing the possible participation of the Ah receptor in experimental systems where genetic polymorphisms in the receptor are not available. SAR studies implicate the Ah receptor in a broad spectrum of biochemical, morphologic, immunologic, neoplastic, and reproductive effects that TCDD and related compounds elicit (Poland and Knutson, 1982; Safe, 1986).

The recent cloning and expression of receptor cDNA should generate important new insights into receptor structure and function in the near future. Receptor cDNA cloning followed the development of an [¹²⁵I]-labeled photoaffinity ligand for the Ah receptor, which provided a method for covalently tagging the receptor protein and permitted the application of denaturing procedures to receptor purification (Poland et al., 1986). Using two-dimensional polyacrylamide gel electrophoresis as a purification technique, Bradfield et al. isolated a small amount of receptor protein and determined the amino acid sequence of its N-terminal domain (Bradfield et al., 1991). The amino acid sequence was used to generate antipeptide antibodies (Poland et al., 1991) and to devise synthetic oligodeoxyribonucleotides, which were used to clone receptor cDNA by library screening (Burbach et al., 1992; Ema et

al., 1992). The deduced amino acid sequence of the C57BL/6 mouse Ah receptor reveals that it has a molecular mass of about 89 kDa with the following features: (1) a basic helix-loop-helix (bHLH) domain, located toward the NH₂ end of the receptor; by analogy with other bHLH systems, the basic region is likely to involve DNA binding, while the helix-loop-helix domain is likely to dimerize with other proteins; (2) a region designated as "PAS," which exhibits sequence homology with Per (a *Drosophila* circadian rhythm protein), Arnt (another protein that contributes to dioxin responsiveness, described below), and Sim (a regulatory protein that participates in *Drosophila* central nervous system [CNS] development); photoaffinity labeling studies suggest that the PAS domain may comprise part of the ligand-binding domain of the receptor; and (3) a glutamine-rich region, which resembles certain "activation domains" present in some other transcription factors; by analogy, this region could interact with coactivator proteins that are yet to be characterized. Thus, like many proteins, the Ah receptor appears to be composed of several different domains, which contribute in different ways to receptor function. It is notable that the Ah receptor does not, by itself, bind strongly to DNA; acquisition of DNA-binding capability appears to require that the receptor interact with another factor (such as the Arnt protein). Thus, the active form of the receptor is heteromeric. The Ah receptor exhibits no obvious structural similarities (such as "zinc finger" DNA-binding domains) to the steroid/thyroid/retinoid family of receptors; therefore, the Ah receptor appears to represent a novel type of ligand-activated transcription factor, distinct from those described previously. Immunohistochemical studies, using antireceptor antibodies, reveal that, in intact mouse hepatoma cells, the unliganded receptor resides in the cytoplasm; exposure of cells to TCDD leads to the accumulation of the receptor within the nucleus. Such observations suggest that the receptor protein may have a domain that controls its movement to the nucleus; however, this remains to be determined.

Human cells contain an intracellular protein whose ligand-binding and hydrodynamic properties resemble those of the Ah receptor in animals (see Cook and Greenlee, 1989; Harris et al., 1989; Lorenzen and Okey, 1991; Roberts et al., 1990; Harper et al., 1991 and references therein, for examples). The human receptor has not been studied extensively, and it is unknown if the properties of the human protein differ substantially from those of the Ah

receptor in animals. Cloning, expression, and functional studies of the human receptor should increase our knowledge of its properties in the near future.

2.4. THE Arnt PROTEIN

Biochemical and hydrodynamic findings suggest that more than one protein participates in the response to TCDD. For example, protein-DNA cross-linking studies imply that the liganded Ah receptor binds to DNA as a heteromeric complex, in partnership with an additional protein(s) (Elferink et al., 1990). In addition, sedimentation experiments (Prokipak and Okey, 1988; Henry et al., 1989), as well as protein-protein cross-linking studies (Gasiewicz et al., 1991; Perdew, 1992), imply that the liganded receptor associates with other proteins in mediating the response to TCDD.

Genetic analyses of mouse hepatoma cells that exhibit diminished responsiveness to TCDD provide evidence that several genes contribute to dioxin action. Chemical selection (resistance to benzo[a]pyrene) and/or physical selection (fluorescence-activated cell sorting) can be used to isolate variant cells that respond poorly to TCDD (Hankinson, 1979; Miller and Whitlock, 1981). The variant phenotypes are stable, and the defects in TCDD responsiveness appear to be mutational in origin (Hankinson, 1981). One type of variant cell exhibits a defect in TCDD binding; it appears to contain an altered Ah receptor. In a second type of variant, TCDD binding is normal; however, the liganded receptors are unable to bind to DNA and fail to accumulate in the nucleus. These variants are defective in a protein termed Arnt (see below). Studies using cell fusion reveal that both variant phenotypes are recessive and that the variants fall into different complementation groups. The latter observation indicates that more than one gene contributes to dioxin responsiveness (Hankinson, 1983; Miller et al., 1983). The data are consistent with the idea that TCDD responsiveness requires both a ligand-binding protein (i.e., the Ah receptor) and a second protein, which mediates the binding of the liganded receptor to DNA.

Hankinson and coworkers used molecular genetic techniques to identify a human cDNA that complements the defect in the second type of variant cell described above. They designated the corresponding protein as Arnt, which stands for Ah receptor nuclear translocator, because of its perceived role in translocating the liganded receptor from the

cytoplasm to the nucleus (Hoffman et al., 1991; Reyes et al., 1992). The human Arnt cDNA encodes a protein of about 86 kDa, which has the following features: (1) like the Ah receptor, it contains a bHLH domain, which presumably contributes both to DNA binding and to protein-protein interactions; (2) like the Ah receptor, the Arnt protein contains domains homologous to Per and Sim. In addition, the Arnt protein does not bind TCDD and it does not bind to DNA in the absence of the liganded Ah receptor protein (Whitelaw et al., 1993). Immunohistochemical studies, using anti-Arnt antibodies, reveal that the Arnt protein resides in the nucleus in uninduced mouse hepatoma cells and that exposure of cells to TCDD produces no change in its intracellular distribution. Thus, Arnt appears to be a nuclear protein. Furthermore, the nuclear accumulation of the Ah receptor occurs in Arnt-defective cells. Together, these findings argue against a primary role for the Arnt protein in the translocation of the receptor from cytoplasm to nucleus per se. Instead, they suggest that Arnt interacts with the liganded Ah receptor to form a heteromeric, DNA-binding protein complex that can activate gene transcription. Experiments in vitro support this idea. For example, immunoprecipitation experiments reveal that the liganded Ah receptor and the Arnt protein can interact in solution. Neither the liganded receptor nor the Arnt protein exhibits substantial DNA-binding activity in the absence of the other; the presence of both proteins is required to generate a specific DNA-binding species and to activate the expression of a reporter gene. Deletion of the bHLH domain of Arnt abrogates its functional interaction with the liganded Ah receptor (Whitelaw et al., 1993). Together, these findings imply that the transcriptionally active component of the dioxin-responsive system is a protein heteromer consisting of (at least) the liganded Ah receptor and Arnt.

Both the Ah receptor and the Arnt protein belong to the bHLH class of transcription factors, which function as heterodimers and contribute to the control of numerous genes (Kadesch, 1993). The dimerization of bHLH proteins constitutes a potential mechanism for generating regulatory diversity. For example, different heterodimers may exhibit different stabilities, may have different DNA-binding affinities, or may recognize different DNA sequences. Its bHLH structure raises the possibility that the Ah receptor might also form heterodimeric complexes with proteins other than Arnt, generating regulatory molecules with potentially novel properties. By analogy with other bHLH systems, both the absolute amount

of each partner and their relative ratios could influence the extent and type of response to TCDD. Thus, diversity in heteromer formation might contribute to the diversity of responses that typifies dioxin action. Furthermore, some HLH proteins (typified by Id), which lack a basic region, may act as dominant negative regulators of transcription by dimerizing with bHLH proteins and inhibiting their binding to DNA (Kadesch, 1993). There is evidence for a dominant negative regulator in the TCDD-responsive system; however, it is not known whether the dominant negative phenotype is due to an HLH protein that interacts with either the Ah receptor or Arnt (Watson and Hankinson, 1988; Watson et al., 1992). In any case, the bHLH structure of the Ah receptor and the Arnt protein suggests that some of the diversity in TCDD's biological effects might reflect differential gene regulation by a combinatorial mechanism involving the formation of different protein heterodimers.

Studies of the Per gene product in vitro by immunoprecipitation suggest that the PAS domain is involved in protein heterodimerization (Huang et al., 1993). By analogy, the PAS domains of the Ah receptor and the Arnt protein may also participate in the formation of specific protein-protein interactions between the protein components of the dioxin-responsive system.

2.5. OTHER PROTEINS THAT PARTICIPATE IN THE RESPONSE TO DIOXIN

Attempts to purify the unliganded Ah receptor under nondenaturing conditions revealed that it tends to associate with other proteins in vitro, particularly the 90-kDa heat shock protein (hsp90). The hsp90 protein is an abundant factor that can interact with numerous other proteins and that may have multiple functions (Welch, 1992).

Immunoprecipitation studies and immunosedimentation experiments using anti-hsp90 antibodies reveal that the unliganded Ah receptor associates with hsp90 in vitro (Denis et al., 1988; Perdew, 1988). In view of previous findings implicating hsp90 in glucocorticoid receptor function (Picard et al., 1990), the association between the Ah receptor and hsp90 in vitro may be more than fortuitous. Experiments in vitro suggest that the hsp90-receptor interaction could have more than one effect; hsp90 might maintain the unliganded receptor in a configuration that facilitates ligand binding, and it might prevent the inappropriate binding of the unliganded receptor to DNA (Pongratz et al., 1992). However, it is unknown whether

hsp90 interacts with the Ah receptor in the intact cell and whether the hsp90 protein influences the response to dioxin *in vivo*. Studies of receptor function in hsp90-defective cells might be instructive in this regard.

Several lines of evidence suggest that phosphorylation/dephosphorylation of the Ah receptor and the Arnt protein may contribute to the function of the dioxin-responsive system. First, treatment of nuclear extracts with potato acid phosphatase inhibits the binding of the liganded receptor heteromer to its DNA recognition sequence *in vitro* (Pongratz et al., 1991). Second, the downregulation of protein kinase C (PKC) and the chemical inhibition of PKC are associated with a reduction in the DNA-binding capability of the receptor heteromer *in vitro* (Okino et al., 1992; Carrier et al., 1992; Berghard et al., 1993). Third, immunoprecipitation experiments using antireceptor antibodies reveal that the receptor can undergo phosphorylation *in vivo* (Berghard et al., 1993). Additional experiments *in vitro* suggest that phosphorylation of the Arnt protein is required for its heterodimerization with the Ah receptor; however, phosphorylation of the receptor is not required for heterodimer formation (Berghard et al., 1993). Together, these findings suggest that PKC and other protein kinases might influence both heterodimerization and the binding of the receptor-Arnt heteromer to DNA. It is known that many mammalian transcription factors undergo cycles of phosphorylation and dephosphorylation; however, in most cases, the physiological significance of the modification is unknown (Hunter and Karin, 1992). Additional research is necessary to delineate fully the role of protein phosphorylation in the response to TCDD.

It appears likely that additional proteins, which remain to be identified and characterized, also contribute to the dioxin-responsive system. For example, a protein phosphatase(s) must participate in the presumed cycles of phosphorylation/dephosphorylation that the Ah receptor and Arnt protein undergo. In addition, the superinduction of CYP1A1 transcription by cycloheximide suggests that an inhibitory gene regulatory protein may modulate the response to dioxin (Israel et al., 1985; Lusska et al., 1992). Chemical cross-linking studies suggest that the Ah receptor contacts multiple proteins as it transduces the TCDD signal to the nucleus (Gasiewicz et al., 1991; Perdew, 1992). Genetic analyses of cells that respond poorly to TCDD indicate the existence of an additional complementation group, whose precise biochemical defect remains to be identified; this observation implies the

existence of another protein (in addition to the Ah receptor and Arnt) that contributes to dioxin responsiveness (Karenlampi et al., 1988).

2.6. ACTIVATION OF GENE TRANSCRIPTION BY DIOXIN

2.6.1. In Vitro Studies

Much of our current understanding of the mechanism of dioxin action is based on analyses of the induction of microsomal aryl hydrocarbon hydroxylase (AHH) activity by TCDD. Hydroxylase activity reflects the action of the cytochrome P450 1A1 enzyme, which catalyzes the oxygenation of polycyclic aromatic substrates, as the initial step in their metabolic processing to water-soluble derivatives (Conney, 1982). TCDD induces AHH activity in many tissues; in particular, the relatively strong induction response in cultured cells has facilitated the application of molecular genetic techniques to the analysis of the induction mechanism (Whitlock, 1990). Table 2-1 and Figure 2-2 summarize some of the molecular events involved in the induction process.

In mouse hepatoma cells, nuclear transcription experiments reveal that TCDD induces hydroxylase activity by stimulating the transcription of the corresponding CYP1A1 gene. The response to TCDD occurs within a few minutes and is direct, in that it does not require ongoing protein synthesis. Thus, the regulatory components required for the activation of CYP1A1 transcription are present constitutively within the cell. TCDD fails to activate CYP1A1 transcription in Ah receptor-defective cells and in Arnt-defective cells; therefore, the response requires both the Ah receptor and the Arnt protein.

The observations that TCDD activates transcription and that the liganded Ah receptor binds to DNA led to the discovery of a dioxin-responsive regulatory DNA domain, upstream of the CYP1A1 gene. Recombinant DNA methods were used to construct chimeric genes, in which potential regulatory DNA domains from the CYP1A1 gene were ligated to a heterologous "reporter" gene. After transfection of the recombinant genes into mouse hepatoma cells, TCDD was observed to activate the expression of the reporter gene. Additional transfection experiments defined the size of the dioxin-responsive domain and revealed that it had the properties of a transcriptional enhancer (Jones et al., 1986; Neuhold

Table 2-1. Events in the Activation of *CYP1A1* Gene Transcription by Dioxin

- Diffusion into the cell
- binding to the Ah receptor protein
- conversion of liganded receptor to the DNA-binding form
 - dissociation from hsp90?
 - active translocation from cytoplasm to nucleus?
 - association with Arnt protein?
- binding of liganded receptor heteromer to enhancer DNA
- enhancer activation
 - altered DNA configuration?
 - histone modification?
 - recruitment of additional proteins?
- nucleosome disruption
- increased accessibility of transcriptional promoter
- binding of transcription factors to promoter
- enhanced mRNA and protein synthesis
- primary biological response(s)
- cascade of compensatory changes?
- secondary biological responses?

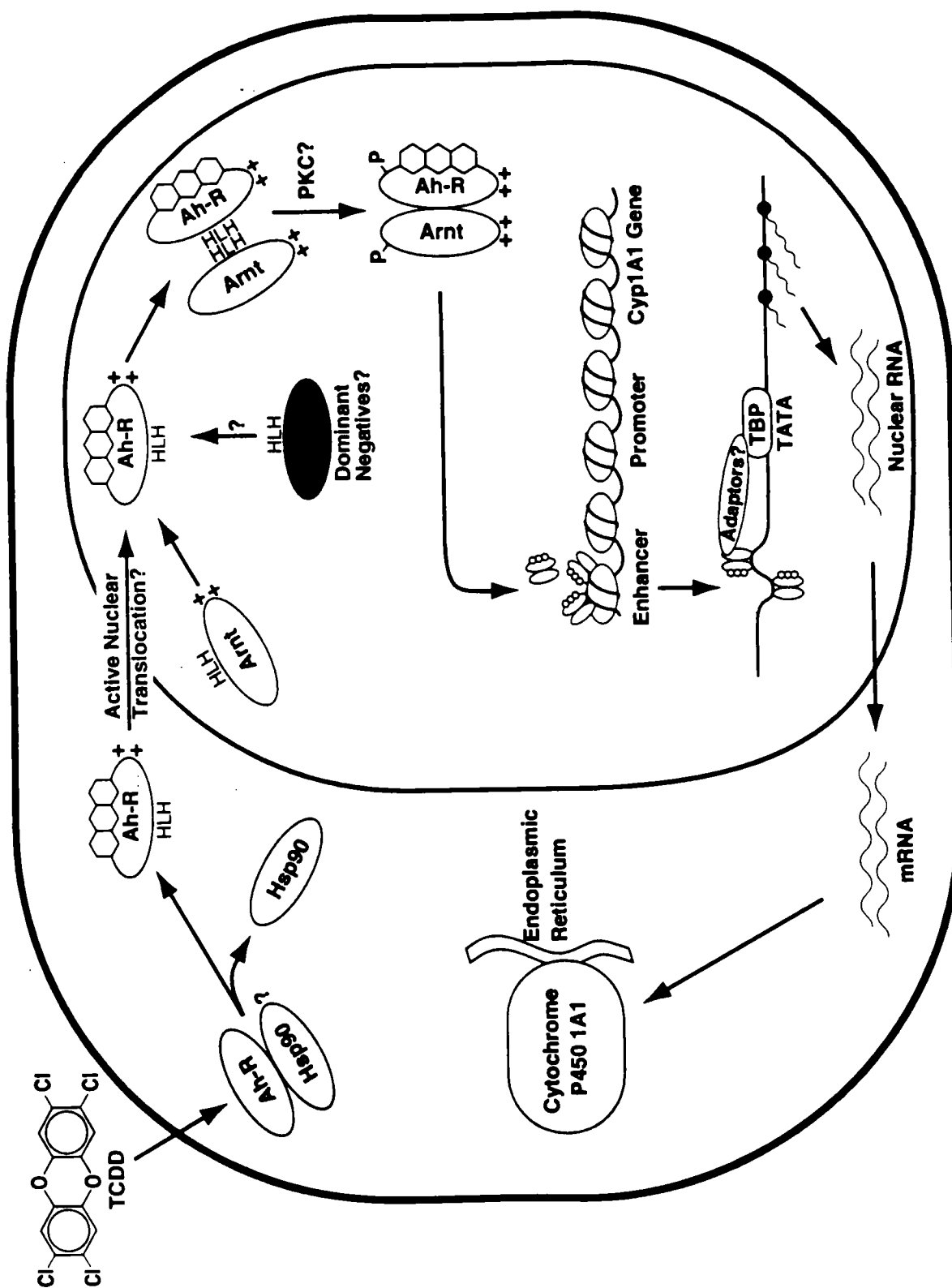


Figure 2-2. Mechanism of induction of CYP1A1 gene transcription by TCDD.

et al., 1986; Fujisawa-Sehara et al., 1987; Fisher et al., 1990). Furthermore, the recombinant gene responded poorly when transfected into Ah receptor-defective cells or Arnt-defective cells. Thus, both the receptor protein and the Arnt protein are required for enhancer function. Analyses of stable transfectants revealed that the dioxin-responsive enhancer can function in a chromosomal location distinct from that of the CYP1A1 gene (Fisher et al., 1989). Therefore, in principle, an analogous enhancer element could mediate the transcriptional response of other genes to TCDD.

In addition, the DNA upstream of the CYP1A1 gene contains a second control element (a transcriptional promoter), which functions to ensure that transcription is initiated at the correct site. The promoter binds proteins that are expressed constitutively by the cell; however, the promoter contains no binding sites for the liganded receptor heteromer. Transfection experiments indicate that neither the enhancer nor the promoter functions in the absence of the other (Jones and Whitlock, 1990). These findings raise the issue as to the mechanism by which the enhancer and promoter, which are separated by hundreds of nucleotides, function in concert. TCDD-induced alterations in the chromatin structure of the CYP1A1 gene appear to play an important role in this process, as described later.

The observations that enhancer function requires both the receptor and Arnt proteins and that the liganded, heteromeric form of the receptor exhibits an increased affinity for DNA suggested that the activation of CYP1A1 transcription involves the binding of the receptor heteromer to the enhancer. Analyses of protein-DNA interactions in vitro by gel retardation, using enhancer DNA and nuclear extracts from uninduced and TCDD-induced cells, revealed the existence of an inducible, receptor-dependent, and Arnt-dependent protein-DNA interaction, whose characteristics were those expected for the binding of the receptor heteromer to DNA (Denison et al., 1989; Hapgood et al., 1989; Saatcioglu et al., 1990a, b). The receptor heteromer recognizes a specific core nucleotide sequence:

5'T-GCGTG 3'

3'A-CGCAC 5'

which is present in multiple copies within the enhancer. Studies with an [^{125} I]-labeled dioxin indicate that the receptor heteromer binds in a 1:1 ratio to its DNA recognition sequence (Denison et al., 1989). Methylation protection and interference experiments in vitro reveal that the receptor heteromer lies within the major DNA groove and contacts the four guanines of the recognition sequence (Saatcioglu et al., 1990a, b; Shen and Whitlock, 1989; Neuhold et al., 1989). Transfection analyses of the six in vivo binding sites for the receptor heteromer as well as several mutated sites synthesized in vitro reveal that base-pairs adjacent to the core recognition sequence contribute to enhancer function. Studies of protein-DNA interactions reveal that there is no strict relationship between the affinity of the receptor heteromer for DNA and the extent of enhancer activation (Shen and Whitlock, 1992; Neuhold et al., 1989). These latter observations suggest that the protein-DNA interaction per se does not suffice to activate transcription and that an additional event (such as DNA bending--see below) is necessary. Mutational analyses reveal that the four base-pair sequence:

5'CGTG 3'
3'GCAC 5'

is required for the receptor heteromer to bind to DNA in vitro (Shen and Whitlock, 1992; Lusska et al., 1993). This sequence is also part of the recognition motif for other bHLH proteins (Kadesch, 1993). This latter observation suggests that the Ah receptor and Arnt proteins may have DNA recognition properties similar to those of some other bHLH proteins.

The DNA recognition sequence for the receptor heteromer contains two CpG dinucleotides. Studies in other systems reveal that cytosine methylation at CpG is associated with decreased gene expression, often in tissue-specific fashion. Cytosine methylation of the CpG dinucleotides within the recognition sequence diminishes both the binding of the receptor heteromer to the enhancer (as measured by gel retardation) and the function of the enhancer (as measured by transfection). Therefore, given that the TCDD-responsive receptor/enhancer system can regulate the transcription of genes other than CYP1A1,

methylation of the enhancer constitutes a mechanism for controlling the expression of such genes in tissue-specific fashion (Shen and Whitlock, 1989).

Gel retardation analyses also reveal that the binding of the receptor heteromer to its recognition sequence bends the DNA in vitro. The site of the bend is at, or very near, the site of the protein-DNA interaction (Elferink and Whitlock, 1990). These findings suggest that the binding of the receptor heteromer to chromatin might also alter the configuration of the enhancer in vivo.

2.6.2. In Vivo Studies

Studies that use transfection and in vitro techniques for analyzing protein-DNA interactions provide important clues about the components of the TCDD-responsive system and their function. However, such experimental approaches necessitate removing the DNA regulatory elements from their native context within the chromosome and, therefore, have the potential to generate misleading results. For example, in the intact cell, nuclear DNA is complexed with histones and other chromosomal proteins, and the structure of the nucleoprotein complex (chromatin) makes important contributions to the control of gene transcription (Grunstein, 1990; Felsenfeld, 1992; Kornberg and Lorch, 1992). Transfection experiments and studies of protein-DNA interactions in vitro do not adequately control for this variable. For this reason, a ligation-mediated polymerase chain reaction technique was employed to analyze the protein-DNA interactions at the dioxin-responsive enhancer in intact cells (Wu and Whitlock, 1993). These experiments reveal that the inactive (i.e., uninduced) enhancer binds few, if any, proteins within the major DNA groove. This finding implies that the inactive enhancer is relatively inaccessible to DNA-binding proteins in vivo. In addition, from a mechanistic standpoint, the absence of protein-enhancer interactions in uninduced cells argues against the idea that a specific repressor protein maintains the enhancer in an inactive configuration. Exposure of cells to TCDD leads to the rapid binding of six receptor heteromers, and few other proteins, to the enhancer. Therefore, the liganded receptor heteromer appears to activate transcription by a mechanism that does not require other enhancer-binding proteins (Wu and Whitlock, 1993).

The proteins that bind to the CYP1A1 promoter are expressed constitutively by the cell, and TCDD has no effect on their interactions with promoter DNA *in vitro*, as measured by DNase footprinting (Jones and Whitlock, 1990). However, in intact cells, these proteins fail to bind to the inactive (i.e., uninduced) promoter. Thus, the promoter, like the enhancer, is inaccessible in uninduced cells. Exposure of cells to TCDD induces a rapid change at the promoter, such that it becomes accessible to the constitutively expressed proteins. The change represents a primary effect of TCDD, because it is insensitive to actinomycin D at a concentration that inhibits transcription by greater than 95%. In addition, it is receptor dependent and Arnt dependent, because it does not occur in the respective variant cells. These observations indicate that the effect of the receptor-enhancer interaction is to increase the accessibility of the downstream promoter (Durrin and Whitlock, 1989; Wu and Whitlock, 1992).

Studies of the chromatin structure of the CYP1A1 gene reveal that, in the transcriptionally inactive state, the enhancer/promoter region assumes a nucleosomal structure and that nucleosomes are specifically positioned at the promoter (Morgan and Whitlock, 1992). Its organization into nucleosomes plausibly accounts for the inaccessibility of the enhancer/promoter region to DNA-binding proteins in uninduced cells. Exposure to TCDD produces a rapid and actinomycin D-insensitive loss of the positioned nucleosomes at the promoter; this change in chromatin structure mechanistically accounts for the TCDD-induced increase in promoter accessibility and increased CYP1A1 transcription *in vivo* (Morgan and Whitlock, 1992).

The mechanism by which the binding of liganded receptor heteromers to the enhancer alters chromatin structure is unknown. One possibility is that the DNA-bound receptor complex affects the histones (for example, by activating a histone acetylase), thereby weakening histone-DNA interactions and destabilizing nucleosomes. A second possibility is that the receptor-enhancer interaction alters the DNA structure of the enhancer/promoter region, stabilizing it in a non-nucleosomal configuration. This idea is consistent with the observation that the receptor heteromer bends the DNA *in vitro* (Elferink and Whitlock, 1990).

On the enhancer, the six binding sites for the receptor heteromer are arranged in an irregular pattern. The absence of regular spacing between the sites suggests that enhancer activation does not require protein-protein interactions between adjacent DNA-bound receptor heteromers. Instead, the irregular spacing of binding sites may reflect constraints imposed by chromatin structure because the receptor heteromer must bind to nucleosomes. For example, as the DNA helix wraps around the histone core of the nucleosome, the major groove (which contains the binding sites for the receptor heteromer) is periodically accessible and inaccessible. Therefore, increasing the number of binding sites at irregular intervals increases the probability that at least one site will be accessible even when the DNA is nucleosomal. In addition, the receptor heteromer contacts a relatively short (six base-pair) DNA segment, increasing the probability that the entire binding site will be accessible in the nucleosome. Thus, the multiplicity, irregular distribution, and small size of the binding sites may have evolved as a mechanism for overcoming the steric constraint imposed by the nucleosomal organization of the inactive enhancer *in vivo*.

2.7. FUTURE RESEARCH

The cloning of cDNAs encoding the Ah receptor and Arnt proteins and the development of antireceptor and anti-Arnt antibodies open the way to additional mechanistic studies of dioxin action. It is now practical to analyze the structure and function of these proteins using mutagenesis techniques, to analyze the structure and regulation of the corresponding genes, to determine whether different forms of the receptor and Arnt exist, and to directly analyze the role of posttranslational modifications on receptor and Arnt function. *In vitro* transcription can be used to study the functional components of the dioxin-responsive pathway (Wen et al., 1990). However, it will be appropriate to develop a chromatin-based system for such studies in the future.

The observation that the Ah receptor and Arnt proteins heterodimerize via HLH motifs raises the likelihood that they may also dimerize with other partners (perhaps in tissue-specific fashion) to generate different combinations of proteins that may have novel regulatory properties. For example, the partial homology between the Ah receptor, Arnt, and Sim suggests that different dimerization partners might contribute to dioxin-induced

alterations in differentiation pathways in some cells. The types of protein complexes formed will presumably depend on both the relative amount of each potential partner and the stability of each type of heterodimer. This combinational mechanism for regulating the response to dioxin may also permit the system to functionally interact with other signal transduction pathways, increasing the potential diversity of dioxin-induced responses still further (see, for example, Pimental et al., 1993). These appear to be interesting areas for future research. The information generated in future experiments is likely to provide novel insights into the mechanism of dioxin action, in particular, and into the regulation of mammalian gene transcription (especially by bHLH proteins), in general. If such studies reveal new proteins (and the corresponding genes) that influence rate-limiting steps in the response to dioxin, the findings might also prove useful from a risk assessment standpoint.

The teratogenic and tumor-promoting effects of TCDD, as well as its effects on keratinocyte differentiation, suggest that the Ah receptor and other components of the dioxin-responsive system may contribute to important developmental and proliferative pathways in some cell types. The study of transgenic animals, in which both alleles for the Ah receptor or the Arnt protein have been inactivated by homologous recombination, might provide new insights into such pathways, assuming that the transgenic animals are viable. In addition, such animals might be useful in toxicological studies to assess whether the product of the inactivated gene participates in adverse responses to particular chemicals.

Chromatin structure and nucleosome positioning have important effects on mammalian gene expression (Grunstein, 1990; Felsenfeld, 1992; Kornberg and Lorch, 1992). The TCDD-responsive CYP1A1 gene is an interesting model system for analyzing the mechanism by which a protein complex such as the liganded receptor heteromer can trigger the chromatin structural changes that increase DNA accessibility. Studies of the triggering mechanism may provide novel insights into the function of the dioxin-responsive system, in particular, and BHLH transcriptional regulatory proteins, in general.

Some dioxin-responsive genes (e.g., cytochrome P450 1A2, glutathione S-transferase Ya) exhibit a substantial constitutive ("basal") transcription, and TCDD increases expression still further. The constitutive expression implies that the promoter for such genes must be maintained in an accessible configuration even in the absence of dioxin; therefore, TCDD

may induce the transcription of such genes by a mechanism that does not involve major changes in the chromatin structure of the regulated gene. Thus, the liganded receptor heteromer may be able to increase gene transcription by both chromatin-dependent and chromatin-independent mechanisms. This may be an interesting issue for future research.

The Ah receptor presumably participates in every biological response to TCDD (Poland and Knutson, 1982; Safe, 1986; Birnbaum, 1993). Thus, it is likely that TCDD activates transcription of other genes via a receptor- and enhancer-dependent mechanism analogous to that described for the CYP1A1 gene. For example, TCDD induces the expression of genes encoding cytochrome P450 1A2, glutathione S-transferase Ya subunit, aldehyde dehydrogenase, and quinone reductase; in some cases, induction is known to occur at the transcriptional level, to be Ah receptor dependent and Arnt dependent, and to involve a DNA recognition sequence analogous to that found upstream of the CYP1A1 gene (Pimental et al., 1993; Quattrochi and Tukey, 1989; Rushmore and Pickett, 1993; Dunn et al., 1988; Takimoto et al., 1992; Favreau and Pickett, 1991). Other observations reveal that, in human keratinocytes, TCDD activates the transcription of the plasminogen activator inhibitor-2 and interleukin-10 genes, as well as additional genes that remain to be identified (Sutter et al., 1991). The mechanism by which dioxin activates keratinocyte gene expression is unknown. For dioxin-responsive genes other than CYP1A1, and especially for those genes that respond in tissue-specific fashion, the presence of the receptor/enhancer system may not be sufficient for dioxin action, and other tissue-specific, regulatory components may play a dominant role in governing the response to TCDD. For example, estrogens (presumably via estrogen receptors) influence TCDD-induced neoplasia in rat liver (Lucier et al., 1991). Also, in vitro experiments suggest the possibility that, in some cell types, a repressor protein might inhibit the response to TCDD by competing for the receptor binding site(s) on DNA (Gradin et al., 1993). Thus, future research may reveal the existence of additional stimulatory or inhibitory gene regulatory components that can modulate the activity of the dioxin-responsive receptor/enhancer system.

The potential teratogenic and neoplastic effects of TCDD have raised particular concerns about the health effects of dioxin in humans. These responses may reflect complicated cascades of biochemical changes that are difficult to analyze mechanistically;

therefore, a major challenge for the future will be to establish experimental systems in which such complex phenomena are amenable to study at the molecular level. However, the identification of a dioxin-transformable human keratinocyte cell line and the development of methods for analyzing dioxin's teratogenic effects in vitro provide reasons to be optimistic about future progress in these areas (Yang et al., 1992; Abbott et al., 1989).

2.8. MECHANISTIC INFORMATION AND RISK ASSESSMENT

A substantial body of evidence in experimental animals indicates that the Ah receptor mediates the biological effects of TCDD. Although studies of human tissue are much less extensive, it appears reasonable to assume that dioxin's effects in humans are also receptor mediated. The receptor-based mechanism predicts that, except in cases where the concentration of TCDD is already very high (i.e., $[TCDD] \gg K_D$), an incremental exposure to TCDD will lead to some increase in the fraction of Ah receptor that is occupied. However, it is not valid to assume that the increased receptor occupancy will necessarily elicit a proportional increase in all biological response(s). Dose-response relationships (which may vary among responses) must be considered when using mathematical models to estimate the risk associated with exposure to TCDD.

Given TCDD's widespread distribution, its persistence, and its accumulation within the food chain, it is likely that most humans are exposed to some level of dioxin; thus, the population at potential risk is large and genetically heterogeneous. By analogy with the findings in inbred mice, polymorphisms in the Ah receptor probably exist in humans. Therefore, a concentration of TCDD that elicits a response in one individual may not do so in another. For example, studies of humans exposed to dioxin following an industrial accident at Seveso, Italy, fail to reveal a simple and direct relationship between blood TCDD levels and the development of chloracne (Mocarelli et al., 1991). These differences in responsiveness to TCDD may reflect genetic variation either in the Ah receptor or in some other component of the dioxin-responsive pathway. Therefore, analyses of polymorphisms in the Ah receptor and Arnt genes in humans with well-documented exposure to dioxin have the potential to identify genotypes that confer particular risk. Such molecular genetic

information may be useful in the future for accurately predicting the health risk that dioxin poses to humans.

Complicated responses (such as cancer) probably involve multiple events and multiple genes. For example, a homozygous recessive mutation at the *hr* (hairless) locus is required for TCDD's action as a tumor promoter in mouse skin (Poland et al., 1982). Thus, the *hr* locus influences the susceptibility of a particular tissue (skin) to a specific effect (tumor promotion) of dioxin. An analogous situation may obtain other effects of TCDD in other tissues. For example, TCDD may produce porphyria cutanea tarda only in individuals with inherited uroporphyrinogen decarboxylase deficiency (Doss et al., 1984). Such findings suggest that, for some adverse effects of TCDD, the population at risk may be limited to individuals with a particular genetic predisposition.

Other factors can influence an organism's susceptibility to TCDD. For example, female rats are more prone to TCDD-induced liver neoplasms than are males; this phenomenon is related to the hormonal status of the animals (Lucier et al., 1991). In addition, hydrocortisone and TCDD synergize in producing cleft palate in mice. Retinoic acid and TCDD produce a similar synergistic teratogenic effect (Couture et al., 1990). Therefore, in some cases, TCDD acts in combination with hormones or other chemicals to produce adverse effects. Such phenomena might also occur in humans; if so, the difficulty of risk assessment is increased, given the diversity among humans in hormonal status, lifestyle (e.g., smoking, diet), and chemical exposure.

Dioxin's action as a tumor promoter presumably reflects its ability to alter cell proliferation. There are several plausible mechanisms by which this could occur. First, TCDD might activate a gene(s) that is directly involved in tissue proliferation. Second, TCDD-induced changes in hormone metabolism may lead to tissue proliferation secondary to increased secretion of a trophic hormone. Third, TCDD-induced changes in receptors for growth factors or hormones may alter the sensitivity of a tissue to proliferative stimuli. Fourth, TCDD-induced toxicity may lead to cell death, followed by regenerative proliferation. The mechanisms might differ among tissues, might exhibit different sensitivities to TCDD, and might be modulated by different genetic and environmental factors. If so, the differences would increase the difficulty of risk assessment.

Under some circumstances, exposure to TCDD elicits beneficial effects. For example, TCDD protects against the carcinogenic effects of polycyclic aromatic hydrocarbons in mouse skin; this may reflect the induction of detoxifying enzymes (Cohen et al., 1979; DiGiovanni et al., 1980). In other situations, TCDD-induced changes in estrogen metabolism may alter the growth of hormone-dependent tumor cells, producing a potential anticarcinogenic effect (Spink et al., 1990; Gierthy et al., 1993). These (and perhaps other) potentially beneficial effects of TCDD further complicate the risk assessment process for dioxin.

Given the diversity of TCDD's biological effects and the likelihood that many effects reflect a complicated interplay between genetic and environmental factors, it may be overly simplistic to use mechanistic information derived largely from the relatively simple responses described in this chapter as the basis for developing a quantitative approach to dioxin risk assessment in humans. While this approach represents a start toward incorporating mechanistic information into risk assessment, future biologically based dose-response models will require a better understanding not only of the TCDD-induced biochemical alterations that produce disease but also of the relationships between genetic and environmental factors that influence an individual's susceptibility to TCDD. Molecular toxicology has great potential to provide new insights into such issues in the future.

REFERENCES

- Abbott, B.D.; Diliberto, J.J.; Birnbaum, L.S. (1989) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin alters embryonic palatal medial epithelial cell differentiation in vitro. *Toxicol. Appl. Pharmacol.* 100: 119-131.
- Bailar, J.C., III. (1991) How dangerous is dioxin? *N. Engl. J. Med.* 324: 260-262.
- Berghard, A.; Gradin, K.; Pongratz, I.; Whitelaw, M.; Poellinger, L. (1993) Cross-coupling of signal transduction pathways: the dioxin receptor mediates induction of cytochrome P4501A1 expression via a protein kinase C mechanism. *Mol. Cell. Biol.* 13: 677-689.
- Birnbaum, L. (1993) Evidence for the role of the Ah receptor in responses to dioxin. *Prog. Clin. Biol. Res.*: in press.
- Bjeldanes, L.F.; Kim, J.Y.; Grose, K.R.; Bartholomew, J.S.; Bradfield, C.A. (1991) Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Proc. Natl. Acad. Sci. U.S.A.* 88: 9543-9547.
- Bradfield, C.A.; Glover, E.; Poland, A. (1991) Purification and N-terminal amino acid sequence of the Ah receptor from the C57BL/6J mouse. *Mol. Pharmacol.* 39: 13-19.
- Burbach, K.M.; Poland, A.; Bradfield, C.A. (1992) Cloning of the Ah-receptor cDNA reveals a novel ligand-activated transcription factor. *Proc. Natl. Acad. Sci. U.S.A.* 89: 8185-8189.
- Carrier, F.; Owens, R.A.; Nebert, D.W.; Puga, A. (1992) Dioxin-dependent activation of murine Cyp1a1 gene transcription requires protein kinase C-dependent phosphorylation. *Mol. Cell. Biol.* 12: 1856-1863.
- Clark, A.J. (1993) The mode of action of drugs on cells. Baltimore, MD: Williams and Wilkins.
- Cohen, G.M.; Bracken, W.M.; Iyer, R.P.; Berry, D.L.; Selkirk, J.K.; Slaga, T.J. (1979) Anticarcinogenic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene tumor initiation and its relationship to DNA binding. *Cancer Res.* 39: 4027-4033.
- Conney, A.H. (1982) Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons. *Cancer Res.* 42: 4875-4917.
- Cook, J.C.; Greenlee, W.F. (1989) Characterization of a specific binding protein for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in human thymic epithelial cells. *Mol. Pharmacol.* 35: 713-719.
- Couture, L.A.; Abbott, B.D.; Birnbaum, L.S. (1990) A critical review of the developmental toxicity and teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: recent advances toward understanding the mechanism. *Teratology* 42: 619-627.
- Czuczwa, J.M.; McVeety, B.D.; Hites, R.A. (1984) Polychlorinated dibenzo-*p*-dioxins and dibenzofurans in sediments from Siskicoit Lake, Isle Royale. *Science* 226: 568-569.
- Denis, M.; Cuthill, S.; Wikstrom, A.C.; Poellinger, L.; Gustafsson, J.-A. (1988) Association of the dioxin receptor with the M_r 90,000 heat shock protein. *Biochem. Biophys. Res. Commun.* 155: 801-807.

DRAFT--DO NOT QUOTE OR CITE

- Denison, M.S.; Fisher, J.M.; Whitlock, J.P., Jr. (1989) Protein-DNA interactions at recognition sites for the dioxin-Ah receptor complex. *J. Biol. Chem.* 264: 16478-16482.
- DiGiovanni, J.; Berry, D.L.; Gleason, G.L.; Kishore, G.S.; Slaga, T.J. (1980) Time-dependent inhibition by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin of skin tumorigenesis with polycyclic hydrocarbons. *Cancer Res.* 40: 1580-1587.
- Doss, M.; Saver, H.; von Tiepermann, R.; Colombi, A.M. (1984) Development of chronic hepatic porphyria (porphyria cutanea tarda) with inherited uroporphyrinogen decarboxylase deficiency under exposure to dioxin. *J. Biochem.* 16: 369-373.
- Dunn, T.J.; Lindahl, R.; Pitot, H.C. (1988) Differential gene expression in response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *J. Biol. Chem.* 263: 10878-10886.
- Durrin, L.K.; Whitlock, J.P., Jr. (1989) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: Ah receptor-mediated change in cytochrome P₁450 chromatin structure occurs independent of transcription. *Mol. Cell. Biol.* 9: 5733-5737.
- Elferink, C.J.; Whitlock, J.P., Jr. (1990) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin inducible, Ah receptor-mediated bending of enhancer DNA. *J. Biol. Chem.* 265: 5718-5721.
- Elferink, C.J.; Gasiewicz, T.A.; Whitlock, J.P., Jr. (1990) Protein-DNA interactions at a dioxin responsive enhancer: evidence that the transformed Ah receptor is heteromeric. *J. Biol. Chem.* 265: 20708-20712.
- Ema, M.; Sogawa, K.; Wanatabe, N.; Chujoh, Y.; Matsushita, N.; Gotoh, O.; Funae, Y.; Fujii-Kuriyama, Y. (1992) cDNA cloning and structure of mouse putative Ah receptor. *Biochem. Biophys. Res. Commun.* 184: 246-253.
- Favreau, L.V.; Pickett, C.B. (1991) Transcriptional regulation of the rat NAD(P)H; quinone reductase gene. *J. Biol. Chem.* 266: 4556-4561.
- Felsenfeld, G. (1992) Chromatin as an essential part of the transcriptional mechanism. *Nature* 355: 219-224.
- Fisher, J.M.; Jones, K.W.; Whitlock, J.P., Jr. (1989) Activation of transcription as a general mechanism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin action. *Mol. Carcinog.* 1: 216-221.
- Fisher, J.M.; Wu, L.; Denison, M.S.; Whitlock, J.P., Jr. (1990) Organization and function of a dioxin-responsive enhancer. *J. Biol. Chem.* 265: 9676-9681.
- Fujisawa-Sehara, A.; Sogawa, K.; Yamane, M.; Fujii-Kuriyama, Y. (1987) Characterization of xenobiotic responsive elements upstream from the drug-metabolizing cytochrome P450c gene: a similarity to glucocorticoid regulatory elements. *Nucleic Acids Res.* 15: 4179-4191.
- Gallo, M.A.; Scheuplein, R.J.; van der Heijden, K.A.; eds. (1991) Banbury report 35: biological basis for risk assessment of dioxins and related compounds. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

DRAFT--DO NOT QUOTE OR CITE

- Gasiewicz, T.A.; Elferink, C.J.; Henry, E.C. (1991) Characterization of multiple forms of the Ah receptor: recognition of a dioxin-responsive enhancer involves heteromer formation. *Biochemistry* 30: 2909-2916.
- Gierthy, J.F.; Bennett, J.A.; Bradley, L.M.; Cutler, D.S. (1993) Correlation of in vitro and in vivo growth suppression of MCF-7 human breast cancer by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Cancer Res.* 53: 3149-3153.
- Gillner, M.; Bergman, J.; Cambillau, C.; Fernstrom, B.; Gustafsson, J.A. (1985) Interactions of indoles with specific binding sites for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rat liver. *Mol. Pharmacol.* 28: 357-363.
- Gillner, M.; Bergman, J.; Cambillau, C.; Gustafsson, J.A. (1989) Interactions of rutaecarpine alkaloids with specific binding sites for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rat liver. *Carcinogenesis* 10: 651-654.
- Gradin, K.; Wilhelmsson, A.; Poellinger, L.; Berghard, A. (1993) Nonresponsiveness of normal human fibroblasts to dioxin correlates with the presence of constitutive xenobiotic response element-binding factor. *J. Biol. Chem.* 268: 4061-4068.
- Grunstein, M. (1990) Histone function in transcription. *Annu. Rev. Cell Biol.* 6: 643-678.
- Hankinson, O. (1979) Single-step selection of clones of a mouse hepatoma cell line deficient in aryl hydrocarbon hydroxylase. *Proc. Natl. Acad. Sci. U.S.A.* 76: 373-376.
- Hankinson, O. (1981) Evidence that benzo(a)pyrene-resistant, aryl hydrocarbon hydroxylase-deficient variants of mouse hepatoma line, Hepa-1, are mutational in origin. *Somatic Cell Genet.* 7: 373-388.
- Hankinson, O. (1983) Dominant and recessive aryl hydrocarbon hydroxylase-deficient mutants of the mouse hepatoma line, Hepa 1, and assignment of the recessive mutants to three complementation groups. *Somatic Cell Genet.* 9: 497-514.
- Hapgood, J.; Cuthill, S.; Denis, M.; Poellinger, L.; Gustafsson, J.A. (1989) Specific protein-DNA interactions at a xenobiotic-responsive element: copurification of dioxin receptor and DNA-binding activity. *Proc. Natl. Acad. Sci. U.S.A.* 86: 60-64.
- Harper, P.A.; Prokipcak, R.D.; Bush, L.E.; Golas, C.L.; Okey, A.B. (1991) Detection and characterization of the Ah receptor in the human colon adenocarcinoma cell line LS 180. *Arch. Biochem. Biophys.* 290: 27-36.
- Harris, M.; Piskorska-Pliszczyńska, J.; Zacharewski, T.; Romkes, M.; Safe, S. (1989) Structure-dependent induction of aryl hydrocarbon hydroxylase in human breast cancer cell lines and characterization of the Ah receptor. *Cancer Res.* 49: 4531-4545.
- Henry, E.B.; Rucci, G.; Gasiewicz, T.A. (1989) Characterization of multiple forms of the Ah receptor: comparison of species and tissues. *Biochemistry* 28: 6430-6440.
- Hoffman, E.C.; Reyes, H.; Chu, F.-F.; Sander, F.; Conley, L.H.; Brooks, B.A.; Hankinson, O. (1991) Cloning of a factor required for activity of the Ah (dioxin) receptor. *Science* 252: 954-958.

DRAFT--DO NOT QUOTE OR CITE

- Huang, Z.J.; Edery, I.; Robash, M. (1993) PAS is a dimerization domain common to Drosophila Period and several transcription factors. *Nature* 364: 259-262.
- Hunter, T.; Karin, M. (1992) The regulation of transcription by phosphorylation. *Cell* 70: 375-387.
- Israel, D.I.; Estolano, M.G.; Galeazzi, D.R.; Whitlock, J.P., Jr. (1985) Superinduction of cytochrome P₁-450 gene transcription by inhibition of protein synthesis in wild type and variant mouse hepatoma cells. *J. Biol. Chem.* 260: 5648-5653.
- Johnson, E.S. (1992) Human exposure to 2,3,7,8-TCDD and risk of cancer. *Toxicology* 21: 451-463.
- Jones, K.W.; Whitlock, J.P., Jr. (1990) Functional analysis of the transcriptional promoter for the CYP1A1 gene. *Mol. Cell. Biol.* 10: 5098-5105.
- Jones, P.B.C.; Durrin, L.K.; Galeazzi, D.R.; Whitlock, J.P., Jr. (1986) Control of cytochrome P1-450 gene expression: analysis of a dioxin-responsive enhancer system. *Proc. Natl. Acad. Sci. U.S.A.* 83: 2802-2806.
- Kadesch, T. (1993) Consequences of heteromeric interactions among helix-loop-helix proteins. *Cell Growth Differ.* 4: 49-55.
- Karenlampi, S.O.; Legraverend, C.; Gudas, J.; Carramanzana, N.; Hankinson, O. (1988) A third genetic locus affecting the Ah (dioxin) receptor. *J. Biol. Chem.* 263: 10111-10117.
- Knutson, J.C.; Poland, A. (1980) Keratinization of mouse teratoma cell line XB produced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: an in vitro model of toxicity. *Cell* 22: 27-36.
- Kornberg, R.D.; Lorch, Y. (1992) Chromatin structure and transcription. *Annu. Rev. Cell. Biol.* 8: 563-587.
- Landers, J.P.; Bunce, N.J. (1991) The Ah receptor and the mechanism of dioxin toxicity. *Biochem. J.* 276: 273-287.
- Lorenzen, A.; Okey, A.B. (1991) Detection and characterization of Ah receptor in tissue and cells from human tonsils. *Toxicol. Appl. Pharmacol.* 107: 203-214.
- Lucier, G.W.; Tritscher, A.; Goldsworthy, T.; Foley, J.; Clark, G.; Goldstein, J.; Marenpot, R. (1991) Ovarian hormones enhance TCDD-mediated increases in cell proliferation and preneoplastic foci in a two stage model for rat hepatocarcinogenesis. *Cancer Res.* 51: 1391-1397.
- Lusska, A.; Wu, L.; Whitlock, J.P., Jr. (1992) Superinduction of CYP1A1 transcription by cycloheximide. *J. Biol. Chem.* 267: 15146-15151.
- Lusska, A.; Shen, E.; Whitlock, J.P., Jr. (1993) Protein-DNA interactions at a dioxin-responsive enhancer: analysis of six bona fide DNA-binding sites for the liganded Ah receptor. *J. Biol. Chem.* 268: 6575-6580.
- Miller, A.G.; Whitlock, J.P., Jr. (1981) Novel variants in benzo(a)pyrene metabolism. *J. Biol. Chem.* 256: 2433-2437.

DRAFT--DO NOT QUOTE OR CITE

- Miller, A.G.; Israel, D.I.; Whitlock, J.P., Jr. (1983) Biochemical and genetic analysis of variant mouse hepatoma cells defective in the induction of benzo(a)pyrene-metabolizing enzyme activity. *J. Biol. Chem.* 258: 3523-3527.
- Mocarelli, P.; Needham, L.L.; Marocchi, A.; Patterson, D.G., Jr.; Brambilla, P.; Gerthoux, P.M.; Meazza, L.; Carreri, V. (1991) Serum concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and test results from selected residents of Seveso, Italy. *J. Toxicol. Environ. Health* 33: 357-366.
- Morgan, J.E.; Whitlock, J.P., Jr. (1992) Transcription-dependent and transcription-independent nucleosome disruption induced by dioxin. *Proc. Natl. Acad. Sci. U.S.A.* 89: 11622-11626.
- Nebert, D.W.; Peterson, D.D.; Puga, A. (1991) Human Ah locus polymorphism and cancer: inducibility of CYP1A1 and other genes by combustion products and dioxin. *Pharmacogenetics* 1: 68-78.
- Neuhold, L.A.; Gonzales, F.J.; Jaiswal, A.K.; Nebert, D.W. (1986) Dioxin-inducible enhancer region upstream from the mouse P1-450 gene and interaction with a heterologous SV40 promoter. *DNA* 5: 403-411.
- Neuhold, L.A.; Shirayoshi, Y.; Ozato, K.; Jones, J.E.; Nebert, D.W. (1989) Regulation of mouse Cyp1A1 gene expression by dioxin: requirement of two cis-acting elements during induction. *Mol. Cell. Biol.* 9: 2378-2386.
- Okino, S.T.; Pendurthi, U.R.; Tukey, R.H. (1992) Phorbol esters inhibit the dioxin receptor-mediated transcriptional activation of the mouse Cyp1a1 and Cyp1a2 genes by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Biol. Chem.* 267: 6991-6998.
- Perdew, G.H. (1988) Association of the Ah receptor with the 90-kDa heat shock protein. *J. Biol. Chem.* 263: 13802-13805.
- Perdew, G.H. (1992) Chemical cross-linking of the cytosolic and nuclear forms of the Ah receptor in hepatoma cell line 1c1c7. *Biochem. Biophys. Res. Commun.* 182: 55-62.
- Picard, D.; Khursheed, B.; Garabedian, M.J.; Fortin, M.G.; Lindquist, S.; Yamamoto, K.R. (1990) Reduced levels of hsp90 compromise steroid receptor action in vivo. *Nature* 348: 166-168.
- Pimental, R.A.; Liang, B.; Yee, G.K.; Wilhelmsson, A.; Poellinger, L.; Paulson, K.E. (1993) Dioxin receptor and C/EBP regulate the function of the glutathione S-transferase Ya gene xenobiotic response element. *Mol. Cell Biol.* 13: 4365-4373.
- Pirkle, J.L.; Wolfe, W.M.; Patterson, D.G., Jr.; Needham, L.L.; Michalek, J.E.; Miner, J.C.; Peterson, M.R.; Phillips, D.L. (1989) Estimates of the half-life of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in Vietnam veterans of Operation Ranch Hand. *J. Toxicol. Environ. Health* 27: 165-171.
- Poellinger, L.; Göttlicher, M.; Gustafsson, J.-A. (1992) The dioxin and peroxisome proliferator-activated receptors: nuclear receptors in search of endogenous ligands. *Trends Pharmacol. Sci.* 13: 241-245.
- Poland, A.; Knutson, J.C. (1982) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related aromatic hydrocarbons: examination of the mechanism of toxicity. *Annu. Rev. Pharmacol. Toxicol.* 22: 517-554.

DRAFT--DO NOT QUOTE OR CITE

- Poland, A.; Palen, D.; Glover, E. (1982) Tumor promotion by TCDD in skin of HRS/J hairless mice. *Nature* 300: 271-273.
- Poland, A.; Glover, E.; Bradfield, C.A. (1991) Characterization of polyclonal antibodies to the Ah receptor prepared by immunization with a synthetic peptide hapten. *Mol. Pharmacol.* 39: 20-26.
- Poland, A.; Glover, E.; Ebetino, F.H.; Kende, A.S. (1986) Photoaffinity labeling of the Ah receptor. *J. Biol. Chem.* 261: 6352-6365.
- Pongratz, I.; Stromstedt, P.-E.; Mason, G.G.F.; Poellinger, L. (1991) Inhibition of the specific DNA binding activity of the dioxin receptor by phosphatase treatment. *J. Biol. Chem.* 266: 16813-16817.
- Pongratz, I.; Mason, G.G.F.; Poellinger, L. (1992) Dual roles of the 90 kDa heat shock protein hsp90 in modulating functional activities of the dioxin receptor. *J. Biol. Chem.* 267: 13728-13734.
- Prokipak, R.D.; Okey, A.B. (1988) Physicochemical characterization of the nuclear form of Ah receptor from mouse hepatoma cells exposed in culture to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Arch. Biochem. Biophys.* 261: 6352-6365.
- Quattrochi, L.C.; Tukey, R.H. (1989) The human cytochrome Cyp1A2 gene contains regulatory elements responsive to 3-methylcholanthrene. *Mol. Pharmacol.* 36: 66-71.
- Rannung, A.; Rannung, U.; Rosenkratz, H.S.; Winqvist, L.; Westerholm, R.; Agurell, E.; Grafstrom, A.K. (1987) Certain photooxidized derivatives of tryptophan bind with very high affinity to the Ah receptor and are likely to be endogenous signal substances. *J. Biol. Chem.* 262: 15422-15427.
- Reyes, H.; Reiz-Porszasz, S.; Hankinson, O. (1992) Identification of the Ah receptor nuclear translocator protein (Arnt) as a component of the DNA binding form of the Ah receptor. *Science* 256: 1193-1195.
- Roberts, E.A.; Johnson, K.C.; Harper, P.A.; Okey, A.B. (1990) Characterization of the Ah receptor mediating aryl hydrocarbon hydroxylase induction in the human liver cell line HepG2. *Arch. Biochem. Biophys.* 276: 442-450.
- Rushmore, T.H.; Pickett, C.B. (1993) Glutathione S-transferases, structure, regulation, and therapeutic implications. *J. Biol. Chem.* 268: 11475-11478.
- Saatcioglu, F.; Perry, D.J.; Pasco, D.S.; Fagan, J.B. (1990) Aryl hydrocarbon (Ah) receptor DNA-binding activity. Sequence specificity and Zn^{2+} requirement. *J. Biol. Chem.* 265: 9251-9258.
- Saatcioglu, F.; Perry, D.J.; Pasco, D.S.; Fagan, J.B. (1990) Multiple DNA-binding factors interact with overlapping specificities at the aryl hydrocarbon response elements of the cytochrome P4501A1 gene. *Mol. Cell. Biol.* 10: 6408-6416.
- Safe, S.H. (1986) Comparative toxicology and mechanism of action of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. *Annu. Rev. Pharmacol. Toxicol.* 26: 371-398.
- Shen, E.S.; Whitlock, J.P., Jr. (1989) The potential role of DNA methylation in the response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Biol. Chem.* 264: 17754-17758.

DRAFT--DO NOT QUOTE OR CITE

- Shen, E.S.; Whitlock, J.P., Jr. (1992) Protein-DNA interactions at a dioxin-responsive enhancer: mutational analysis of the DNA-binding site for the liganded Ah receptor. *J. Biol. Chem.* 267: 6815-6819.
- Silbergeld, E.K.; Gasiewicz, T.A. (1989) Dioxins and the Ah receptor. *Am. J. Ind. Med.* 16: 455-474.
- Skene, S.A.; Dewhurst, I.C.; Greenberg, M. (1989) Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans: the risks to human health. A review. *Hum. Toxicol.* 8: 173-203.
- Spink, D.C.; Lincoln, D.C., II; Dickerman, H.W.; Gierthy, J.F. (1990) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin causes an extensive alteration of 17 β -estradiol metabolism in MCF-7 breast tumor cells. *Proc. Natl. Acad. Sci. U.S.A.* 87: 6917-6921.
- Stephenson, R.P. (1956) A modification of receptor theory. *Br. J. Pharmacol.* 11:379.
- Sutter, T.R.; Cuzman, K.; Dold, K.M.; Greenlee, W.F. (1991) Targets for dioxin: genes for plasminogen activator inhibitor-2 and interleukin-1 β . *Science* 254: 415-518.
- Takimoto, K.; Lindahl, R.; Pitot, H.C. (1992) Regulation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin inducible expression of aldehyde dehydrogenase in hepatoma cells. *Arch. Biochem. Biophys.* 298: 492-497.
- Watson, A.J.; Hankinson, O. (1988) DNA transfection of a gene repressing aryl hydrocarbon hydroxylase induction. *Carcinogenesis* 9: 1581-1586.
- Watson, A.J.; Weir-Brown, K.I.; Bannister, R.M.; Chu, F.-F.; Reinz-Porszasz, S.; Fujii-Kuriyama, Y.; Sogawa, K.; Hankinson, O. (1992) Mechanism of action of a repressor of dioxin-dependent induction of Cyp1a1 gene transcription. *Mol. Cell. Biol.* 12: 2115-2123.
- Welch, W.J. (1992) Mammalian stress response: cell physiology structure/function of stress proteins, and implications for medicine and disease. *Physiol. Rev.* 72: 1063-1081.
- Wen, L.P.; Koeiman, N.; Whitlock, J.P., Jr. (1990) Dioxin-inducible, Ah receptor-dependent transcription in vitro. *Proc. Natl. Acad. Sci. U.S.A.* 87: 8545-8549.
- Whitelaw, M.; Pongratz, I.; Wilhelmsson, A.; Gustafsson, J.-A.; Poellinger, L. (1993) Ligand-dependent recruitment of the Arnt coregulator determines DNA recognition by the dioxin receptor. *Mol. Cell. Biol.* 13: 2504-2514.
- Whitlock, J.P., Jr. (1990) Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin action. *Annu. Rev. Pharmacol. Toxicol.* 30: 251-277.
- Wu, L.; Whitlock, J.P., Jr. (1992) Mechanism of dioxin action: Ah receptor-mediated increase in promoter accessibility in vivo. *Proc. Natl. Acad. Sci. U.S.A.* 89: 4811-4815.
- Wu, L.; Whitlock, J.P., Jr. (1993) Mechanism of dioxin action: receptor-enhancer interactions in intact cells. *Nucleic Acids Res.* 21: 119-125.
- Yang, J.; Thraves, P.; Dritschilo, A.; Rhim, J.S. (1992) Neoplastic transformation of immortalized human keratinocytes by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Cancer Res.* 52: 3478-3482.

Chapter 3. Acute, Subchronic, and Chronic Toxicity

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

NOTICE

THIS DOCUMENT IS A PRELIMINARY DRAFT. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

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

DRAFT--DO NOT QUOTE OR CITE

DISCLAIMER

This document is an internal draft for review purposes only and does not constitute Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

3. ACUTE, SUBCHRONIC, AND CHRONIC TOXICITY

3.1. SCOPE AND LIMITATIONS

The acute, subchronic, and chronic toxicology of the chlorinated dioxins, dibenzofurans, biphenyls, and related compounds has been reviewed extensively in recent years [CDDs and CDFs, WHO/IPCS (1989), U.S. EPA (1984, 1985); PCBs and PCTs, WHO/IPCS (1991); U.S. EPA (1990); PCBs, U.S. EPA (1990); and BDDs and BDFs, U.S. EPA (1991)]. This chapter is intended to summarize our knowledge of the toxicology of TCDD in the main, but includes references to other dioxinlike compounds when relevant data are available. In this chapter, we do not reference all published material but rather have selected various data that are considered to be of importance to risk assessment. The chapter covers experimental animal data. Immunotoxicity, reproductive/developmental toxicity, carcinogenicity, toxicity to humans, and epidemiology are all dealt with in separate chapters. Ecotoxicology also is not covered in this chapter.

3.2. ACUTE TOXICITY

The range of doses of TCDD that are lethal to animals varies extensively with both species and strain, as well as with sex, age, and the route of administration within a single strain (Table 3-1). Typically there is a delayed toxicity, with the time to death after exposure usually being several weeks. However, deaths within the first week after exposure have been observed in guinea pigs (Schwetz et al., 1973), rabbits (Schwetz et al., 1973), and Golden Syrian hamsters (Olson et al., 1980). A more than 8,000-fold difference exists between the dose of TCDD reported to cause 50 percent lethality in male Hartley guinea pigs, the most sensitive species tested (Schwetz et al., 1973), and the corresponding dose in male Syrian Golden hamsters (Henck et al., 1981). Another animal with extremely high sensitivity is the mink (*Mustela vison*); for the male, an LD₅₀ value of only 4.2 µg/kg has been calculated (Hochstein et al., 1988).

TABLE 3-1

Acute Lethality of TCDD to Various Species and Substrains

Species/Strain/Sex	Route	LD ₅₀ (µg/kg)	Time of Death (days post-exposure)	Follow-up (days)	Body Weight Loss ^a (%)	References
Guinea pig/Hartley (male)	<i>per os</i>	0.6-2.1	5-34	NR 30	50	McConnell et al., 1978b; Schwetz et al., 1973
Mink/NR (male)	<i>per os</i>	4.2	7-17	28	31	Hochstein et al., 1988
Chicken/NR (NR)	<i>per os</i>	<25	12-21	NR	NR	Greig et al., 1973
Monkey/rhesus (female)	<i>per os</i>	~70	14-34	42-47	13-38	McConnell et al., 1978a
Rat/L-E (male)	intraperitoneal	~10	15-23	48-49	39	Tuomisto and Pohjanvirta, 1987
Rat/Sherman, Spartan (male) (female)	<i>per os</i>	22 13-43	9-27	NR	NR	Schwetz et al., 1973
Rat/Sprague-Dawley (male) (female) (weanling male)	intraperitoneal	60 25 25	NR	20	NR	Beatty et al., 1978
Rat/Fischer Harlan (male)	<i>per os</i>	340	28 ^b	30	43	Walden and Schiller, 1985
Rat/H/W (male)	intraperitoneal	>3000	23-34	39-48	40-53	Pohjanvirta and Tuomisto, 1987; Pohjanvirta et al., 1988
Mouse/B6 (male) D2A/2J (male) B6D2F1 (male)	<i>per os</i>	182 2570 296	24 ^b 21 ^b 25 ^b	30	25 33 34	Chapman and Schiller, 1985
Mouse/B6 D2 B6D2F1	intraperitoneal	132 620 300	NR	NR	NR	Neal et al., 1982
Rabbit/New Zealand White (male and female)	<i>per os</i> dermal	115 275	6-39 12-22	NR 22	NR NR	Schwetz et al., 1973

TABLE 3-1 (cont.)

Species/Strain/Sex	Route	LD ₅₀ (μ g/kg)	Time of Death (days post-exposure)	Follow-up (days)	Body Weight Loss ^a (%)	References
Rabbit/New Zealand White (male and female)	intraperitoneal	~ 50	7-10	10-20	11	Brewster et al., 1988
Hamster/Golden Syrian (male and female)	<i>per os</i>	1157-5051	2-47	50	NR	Henck et al., 1981
Hamster/Golden Syrian (male and female)	intraperitoneal	> 3000	14-32	55	1 ^c	Olson et al., 1980

^aOf succumbed animals^bMean time to death^cData from five animals

NR = Not reported

DRAFT--DO NOT QUOTE OR CITE

Among traditional experimental animals, the rat seems to be the second most sensitive species, although there is a >300-fold variability in LD₅₀ values among different strains. The Han/Wistar (Kuopio) strain of rat has been shown to be particularly resistant to TCDD exposure (Pohjanvirta and Tuomisto, 1987). Among the five rats per dose group (0, 1,500, 2,000, 2,500 or 3,000 µg TCDD/kg bw), only one animal died within the 39-40 days observation period. Also, the DBA/2 male mouse has been shown to have a high resistance to TCDD toxicity (Chapman and Schiller, 1985).

Data on sex differences in sensitivity to the lethal effects of TCDD are conflicting. Acute toxicity data that address the effect of age at the point of exposure to TCDD are scarce, and comparisons are hampered by either the absence or the inadequacy of the information on the age and/or body weight of the tested animals. Additionally, as demonstrated with other chemicals, the acute toxicity may vary several fold, depending on the vehicle used or the presence of other substances that affect uptake.

The differences in sensitivity toward TCDD among various strains of mice have been claimed to depend on a genetic variability in the Ah locus (see Chapter 2). In two strains of male C57B/6J mice that differ only at the Ah locus, Birnbaum et al. (1990) found LD₅₀ values of 159 and 3,351 µg/kg for the wild-type mice (Ah^{b/b}) and the congenic mice (Ah^{d/d}), respectively. The mean time to death was 22 days and was independent of dose and genotype. Signs of toxicity were similar in the two strains, and it was concluded that the spectrum of toxicity is independent of the allele at the Ah locus. However, the relative dose needed to bring about various acute responses is ~8-24 times greater in congenic mice homozygous for the "d" allele than in the wild-type mice carrying two copies of the "b" gene.

In contrast, the two strains of rats, Long-Evans and Han/Wistar (Kuopio), studied by Pohjanvirta et al. (1988) had intraperitoneal LD₅₀ values of 10 and >3,000 µg TCDD/kg, respectively, although no differences with regard to the amount or the affinity of available Ah receptor could be found.

DRAFT--DO NOT QUOTE OR CITE

Geyer et al. (1990) utilized both their own and other data to determine a correlation between total body fat content and acute toxicity in various species and strains of laboratory mammals. They found a correlation of 0.834 and suggested that the reason for this correlation was that an increased total body fat content may enhance the capacity to remove TCDD from the systemic circulation. This factor may be important, but it almost certainly does not explain all the interspecies differences. Data from studies of the Han/Wistar (Kuopio) rats, which are extremely resistant to TCDD-induced lethality (Pohjanvirta and Tuomisto, 1987), were not included.

In chickens, acute toxicity is characterized by clinical signs such as dyspnea, reduced body weight gain, stunted growth, subcutaneous edema, pallor, and sudden death (chick edema disease). The disease first gained attention in 1957, but the causal agents were not identified as CDDs until much later (Firestone, 1973). Chick edema occurred in birds given oral doses of 1 or 10 μg TCDD/kg/day or of 10 and 100 μg hexaCDD/kg/day, but it was not observed in chicks maintained on a diet containing 0.1 or 0.5 percent OCDD (Schwetz et al., 1973).

3.2.1. Signs and Symptoms of Toxicity

TCDD affects a variety of organ systems in different species. It should be noted that much of the comparative data base is derived from high-dose effects. The liver is the organ primarily affected in rodents and rabbits, while in guinea pigs, atrophy of the thymus and lymphatic tissues seems to be the most sensitive marker of toxicity (WHO/IPCS, 1989; U.S. EPA, 1984, 1985). It is not possible to specify a single organ whose dysfunction accounts for the lethality. Dermal effects are prominent signs of toxicity in subhuman primates, and changes in epithelial tissues dominate both cutaneously and internally. This is most apparent in nonhuman primates in which the TCDD-induced cutaneous lesions closely mimic the chloracne and hyperkeratosis observed in humans. The histopathological alterations observed

DRAFT--DO NOT QUOTE OR CITE

in epithelial tissues include hyperplastic and/or metaplastic alterations, as well as hypoplastic responses. The toxic responses of various species to TCDD are summarized in Table 3-2 (WHO/IPCS, 1989).

Loss of body weight (wasting syndrome) is a characteristic sign observed in most animals given a lethal dose of TCDD. The weight loss usually manifests itself within a few days after exposure and results in a substantial reduction of the adipose (Peterson et al., 1984) and muscle tissue (Max and Silbergeld, 1987) observed at autopsy. With sublethal doses of TCDD, a dose-dependent decrease in body weight gain occurs.

The greatest species-specific differences in toxicity concern pathological alterations in the liver. Administering lethal doses to guinea pigs does not result in liver damage comparable to the liver lesions observed in rabbits and rats or to the liver changes observed in mice (McConnell et al., 1978b; Moore et al., 1979; Turner and Collins, 1983). In the hamster, manifest liver lesions do not occur even after fatal doses of TCDD; however, the ED₅₀ for increased hepatic weight is only ~15 ug/kg (Gasiewicz et al., 1986). Liver-related enzyme activities in serum are elevated in those animal species where liver damage is a prominent sign of TCDD toxicity. In those animal species where hepatotoxicity is not as apparent, such as monkeys and guinea pigs, these enzyme activities are nearly normal.

Thymic atrophy has also been found in all animal species given lethal doses of TCDD. Treatment of animals with TCDD inhibits bone marrow hematopoiesis in mice, both in vivo and in vitro, by directly altering the colony growth efficiency of stem cells (Chastain and Pazdernik, 1985; Luster et al., 1980, 1985).

Among other signs and symptoms that have been demonstrated in various species, the following should be noted: hepatic porphyria, hemorrhages in various organs, testicular atrophy, reduced prostate weight, reduced uterine weight, increased thyroid weight, lesions of the adrenal glands, inhibited bone marrow hematopoiesis, decreased serum albumin, and increased serum triglycerides and free fatty acids. The details of all underlying studies for these observations have been extensively reviewed (U.S. EPA, 1984, 1985; WHO/IPCS, 1989).

TABLE 3-2

Toxic Response Following Exposure to 2,3,7,8-TCDD: Species Differences^a

Response	Monkey	Guinea Pig	Cow ^b	Rat	Mouse	Rabbit ^b	Chicken ^b	Hamster
HYPERPLASIA AND/OR METAPLASIA								
Gastric mucosa	++	0	+	0	0			0
Intestinal mucosa	+							++
Urinary tract	++	++	++	0	0			
Bile duct and/or gall bladder	++	0	+		++			0
Lung: focal alveolar				++				
Skin	++	0	+ ^c	0	0	++		0
HYPOPLASIA, ATROPHY OR NECROSIS								
Thymus	+	+	+	+	+		+	+
Bone marrow	+	+			±		+	
Testicle	+	+		+	+			
OTHER RESPONSES								
Liver lesions	+	±	++	+	++	+	+	±
Porphyrin	0	0		+	++		+	0
Edema	+	0		0	+		++	+

^aSources: Allen and Lalich, 1962; Allen et al., 1977; Henck et al., 1981; Kimmig and Schultz, 1957; Kociba et al., 1978, 1979; McConnell, 1980; McConnell et al., 1978a,b; Moore et al., 1979; Norback and Allen, 1973; Olson et al., 1980; Schwetz et al., 1973; Turner and Collins, 1983; Vos et al., 1973; Vos and Beems, 1971; Vos and Koeman, 1970.

^bResponses followed exposure to 2,3,7,8-TCDD or structurally related chlorinated hydrocarbons.

^cSkin lesions in cattle have been observed, but they differ from the skin lesions observed in other species.

0 = lesion not observed, + = lesion observed (number of "+" denotes severity), ± = lesion observed to a very limited extent, blank = no evidence reported in literature.

DRAFT--DO NOT QUOTE OR CITE

Effects on heart muscle have also been observed in guinea pigs and rats (Brewster et al., 1987; Kelling et al., 1987; Canga et al., 1988). Five days after a single dose of TCDD (10 $\mu\text{g/kg}$ intraperitoneally) was administered, a significantly decreased beta-adrenergic responsiveness was observed in the right ventricular papillary muscle of the guinea pig (Canga et al., 1988). In the TCDD-treated animals, a decrease in the positive inotropic effects of isoproterenol at 0.03-0.3 μM , but not at 0.1-10 nM, was also demonstrated. Additionally, the responsiveness to low-frequency stimulation and increases in extracellular calcium was enhanced in these animals. Based on these findings, the authors suggest that the heart may be a major target for TCDD toxicity.

In the monkey, several additional symptoms have been registered, such as periorbital edema, conjunctivitis, and thickening of the meibomian glands, followed by loss of the eyelashes, facial hair, and nails (McConnell et al., 1978a). These are symptoms similar to those observed in cases of human intoxication (e.g., occupational exposure, the Seveso incident, and the Yusho and Yu-Cheng toxic oil intoxications (the latter involving exposure to PCBs and CDFs; see Chapter 1).

3.2.2. Studies In Vitro

Over 30 cell types, including primary cultures and cells from established and transformed cell lines derived from various tissues of at least six animal species, have been examined for their general cellular responses to TCDD (Beatty et al., 1975; Knutson and Poland, 1980a; Niwa et al., 1975; Yang et al., 1983a). The effects studied were changes in viability, growth rate, and morphology. Overall, there have been few or no effects documented.

However, other in vitro studies using more specific endpoints of toxicity have clearly indicated effects of TCDD at comparatively low concentrations. Thus, several studies have shown that TCDD affects cultured epidermal keratinocytes through interactions with differentiation mechanisms and that this effect may be regulated by the modulation of epidermal growth factor (EGF) binding to the cells (Hudson et al., 1986). Additionally, in

epithelial cells of human origin, TCDD has been shown to alter differentiation (Hudson et al., 1985), while aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin O-deethylase (EROD) activity have been induced in vitro (see Section 3.5.4).

Wiebel et al. (1991) have identified a cell line (H4IIEC3-derived 5L hepatoma cells) that responds with decreased proliferation at low TCDD concentrations. Thus, half-maximum inhibition of proliferation occurs at a concentration of 0.1-0.3 nM, and the onset of the effect is fairly rapid, manifesting itself as early as 4-8 hours after treatment. Further studies have also demonstrated that insensitive variants of this cell line were deficient in cytochrome P-4501A1 activity and lacked measurable amounts of the Ah receptor (Göttlicher et al., 1990). In addition, 3,3',4,4'-TCB inhibited proliferation in the sensitive cell line, albeit at higher concentrations.

3.2.3. Appraisal

The numerous studies of acute toxicity in various species have demonstrated dramatic species- and strain-specific differences in sensitivity. However, the spectrum of symptoms is generally the same, although species differences exist.

Lethality is typically delayed by several weeks, and there is a pronounced wasting syndrome in almost all laboratory animals. Studies in congenic mice differing in their Ah responsiveness indicate that the sensitivity to acute toxicity of TCDD segregates with the Ah locus. Furthermore, studies of other CDDs, CDFs, and coplanar PCBs demonstrate that the potency for inducing lethality correlates with their ability to bind to the Ah receptor. In contrast, studies in various other species, as well as in various strains of rats, have demonstrated a wide range of sensitivities regardless of rather comparable levels of the Ah receptor.

3.3. SUBCHRONIC TOXICITY

The available studies on the subchronic toxicity of TCDD have been reviewed by the U.S. EPA (1984, 1985) and WHO/IPCS (1989). Overall, the signs and symptoms observed are in agreement with those observed after administration of single doses.

The study of Kociba et al. (1976) is of special interest as it has been used for comparisons of the relative toxicities of other CDDs and CDFs (Plüess et al., 1988 a and b). Adult male and female SD rats, in groups of 12, were given 0, 0.001, 0.01, 0.1, and 1.0 μg TCDD/kg bw by gavage 5 days/week for 13 weeks. At the end of the treatment period, five rats of each sex were sacrificed for histopathological examination. The remaining animals were observed for postexposure effects. The highest dose caused five deaths among the females, three during the treatment period and two after, while two deaths occurred in males in the posttreatment period. The rats given 0.01 μg TCDD/kg did not differ from the controls except for a slight increase in the mean liver-to-body weight ratio.

A 13-week dietary study in SD rats given 1,2,3,4,8-PeCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, or 1,2,3,6,7,8-HxCDF demonstrated that both the subchronic toxicity and depletion of hepatic vitamin A followed the rank order of the ability of the compounds to bind to the Ah receptor (to cause induction of AHH) (Plüess et al., 1988a and b; Håkansson et al., 1990). However, the direct comparisons of the effects are hampered by the differences in toxicokinetic behavior of the compounds. Slightly different relationships with regard to toxicity were obtained in a tumor promotion study, where an initial loading dose (subcutaneous) of 2,3,4,7,8-PeCDF was given, followed by repeated lower doses (subcutaneous) in order to obtain a steady-state concentration (Wærn et al., 1991a). However, both of these studies support the assumption that most signs and symptoms obtained may be mediated through the Ah receptor.

In another study, groups of eight female SD rats were exposed to 16 weekly oral doses of 0, 0.01, 0.1, 1.0, and 10.0 μg TCDD/kg bw in a study primarily aimed at investigating TCDD-induced porphyria (Goldstein et al., 1982). The no-effect dose for porphyria was 0.01 $\mu\text{g}/\text{kg}/\text{week}$.

DRAFT--DO NOT QUOTE OR CITE

Only two studies of limited value have been performed in mice (Harris et al., 1973; Vos et al., 1973). Four weekly oral doses of 0.2, 1, 5, or 25 μg TCDD/kg bw were given to male C57Bl/6 mice in corn oil. No effects were noted at 1 μg , which corresponds to ~ 0.1 $\mu\text{g}/\text{kg}$ bw/day.

In male and female Hartley guinea pigs, a 90-day feeding study of TCDD was performed by DeCaprio et al. (1986) where the surviving animals were subjected to extensive pathologic, hematologic, and serum chemical analyses. The diets contained 0, 2, 10, 76, or 430 ng TCDD/kg^{bw}. The two lowest doses, 2 and 10 ng/kg of diet, produced no dose-related alterations. Based on this study a no-observed-effect level of 0.6 ng TCDD/kg bw/day in guinea pigs was estimated. At the highest dose, severe body weight losses and mortality were observed. No dose-related mortality occurred at 76 ng/kg.

A cumulative dose of 0.2 μg TCDD/kg bw, which was divided into nine oral doses 3 times/week during days 20–40 of gestation, produced no clinical signs of toxicity in pregnant rhesus monkeys (*Macaca mulatta*) (McNulty, 1984). However, signs of toxicity such as body weight loss, epidermal changes, and anemia did occur in those monkeys who had received cumulative doses of 1.0 and 5.0 μg TCDD/kg bw over the same time period.

3.3.1. Appraisal

Utilizing the above data, subchronic NOAELs for rats, mice, and guinea pigs are estimated to be 0.01 μg , 0.1 μg , and 0.6 ng TCDD/kg bw/day, respectively. However, these studies cannot be directly compared with each other. Furthermore, none of the studies utilized initial loading doses, and due to the long half-life of TCDD, steady states may not have been reached in the animals except at the very end of the study periods. Distribution between tissues in the animals depends on both time of exposure and dose level (see Chapter 1), which further complicates any comparisons.

In spite of this, the limited data available seem to indicate that signs and symptoms of subchronic toxicity follow the same rank order as Ah receptor-mediated effects, such as induction of AHH.

3.4. CHRONIC TOXICITY

The results of chronic toxicity studies performed on laboratory animals exposed to TCDD are summarized in Table 3-3. Details have been reviewed by the U.S. EPA (1984, 1985) and WHO/IPCS (1989).

The most important study in rats is the chronic toxicity study of Kociba et al. (1978, 1979). Groups of 50 male and 50 female SD rats were fed diets providing daily doses of 0.001, 0.01, and 0.1 μg TCDD/kg bw for 2 years. Control rats, 86 males and 86 females, received diets containing the vehicle alone. Increased mortality was observed in females given 0.1 $\mu\text{g}/\text{kg}/\text{day}$, while increased mortality was not observed in male rats at this dose or in animals receiving doses of 0.01 or 0.001 $\mu\text{g}/\text{kg}/\text{day}$. From month 6 to the end of the study, the mean body weights of males and females decreased at the highest dose and, to a lesser degree, in females given 0.01 $\mu\text{g}/\text{kg}/\text{day}$. During the middle of the study, lower-than-normal body weights were also occasionally recorded in the low-dose group, although during the last quarter of the study, the body weights were comparable with those of the controls.

Increased urinary coproporphyrin and uroporphyrin were noted in females, but not in males, given TCDD at a dose rate of 0.01 and 0.1 $\mu\text{g}/\text{kg}/\text{day}$. Analyses of blood serum collected at terminal necropsy revealed increased enzyme activities related to impaired liver function in female rats given 0.1 μg TCDD/kg/day. Necropsy examination of the rats surviving TCDD exposure until the end of the study revealed that effects in the liver constituted the most consistent alteration in both males and females. Histopathological examination revealed multiple degenerative, inflammatory, and necrotic changes in the liver that were more extensive in females. Multinucleated hepatocytes and bile-duct hyperplasia were also noted. Liver damage was dose related, and no effect was observable at the low-dose rate. The NOAEL was estimated to be 0.001 $\mu\text{g}/\text{kg}/\text{day}$. At the end of the study, the fat and liver concentration of TCDD at this dose was 540 ppt.

In male Swiss mice, weekly oral doses of 0, 0.007, 0.7, and 7.0 μg TCDD/kg bw for 1 year resulted in amyloidosis and dermatitis (Toth et al., 1979). The incidence of these lesions was 0 of 38, 5 of 44, 10 of 44, and 17 of 43 in the control-, low-, medium-, and

TABLE 3-3

Studies on Chronic Exposure (Except for Studies on Cancer) to TCDD in Laboratory Animals

Species/Strain	Sex and No. per Group	Doses Tested	Treatment Schedule	Parameters Monitored	References
Rats/Sprague-Dawley	M/10	0, 1, 5, 50, 500, 1000, 5000, 50,000, 500,000, 1,000,000 ppt	continuous in diet for 65 weeks	survival	Van Miller et al., 1977
Rats/Sprague-Dawley	M, F/10	0.001, 0.01, 0.1 $\mu\text{g/kg/day}$	continuous in diet for 2 years	extensive histopathology, hematology and clinical chemistry	Kociba et al., 1978, 1979
Mice/Swiss	M/38-44	0, 0.007, 0.7, 7.0 $\mu\text{g/kg/week}$	gavage weekly for 1 year	histopathology	Toth et al., 1979
Mice/B6C3F1	M/50, F/50 M/75, F/75	0.01, 0.05, 0.5 $\mu\text{g/kg/week}$ (males) 0.04, 0.2, 2.0 $\mu\text{g/kg/week}$ (females) 0.0	gavage biweekly for 2 years	extensive histopathology	NTP, 1980
Monkey/Macaca mulatta	F/8	500 ppt	continuous in the diet for 9 months	extensive histopathology, hematology and clinical chemistry	Allen et al., 1977

DRAFT--DO NOT QUOTE OR CITE

high-dose groups, respectively. The LOAEL in this study was estimated to be 0.001 $\mu\text{g/kg/day}$.

In the NTP (1980) gavage study in B6C3F1 male and female mice, no adverse effects were seen at the lowest dose tested (i.e., 0.01 and 0.04 $\mu\text{g/kg bw/week}$ for males and females, respectively, corresponding to ~ 1.4 and 6 ng/kg bw/day).

The limited studies (9-20 months) available in rhesus monkeys (Allen et al., 1977; Barsotti et al., 1979; Schantz et al., 1978) revealed signs and symptoms similar to those recorded in more short-term studies. Adverse effects were noted down to the lowest dose tested (i.e., $\sim 2\text{-}3$ ng/kg bw/day for 20 months) (Schantz et al., 1978).

3.4.1. Appraisal

From the different long-term studies on TCDD, it can be estimated that the NOAEL for the rat is 1 ng/kg bw/day , corresponding to a fat and liver concentration (NOEL) of 540 ppt. For the male Swiss mouse, effects (dermatitis and amyloidosis in 5 of 44 animals) were noted at the lowest dose tested (i.e., the LOEL would be 1 ng/kg bw/day). However, in B6C3F1 mice, NOELs of 1.4 and 6 ng/kg/day were obtained for males and females, respectively. The studies in the rhesus monkey cannot be used for such a determination. Adverse effects were observed at the lowest dose tested, $\sim 2\text{-}3$ ng/kg body weight .

3.5. SPECIFIC EFFECTS

3.5.1. Wasting Syndrome

TCDD at high doses (lethal or near lethal) causes a starvationlike or wasting syndrome in several animal species. In young animals or following a sublethal dose to adults, this response is manifested as a cessation of weight gain. Animals exposed to near lethal or higher doses characteristically lose weight rapidly. Numerous studies utilizing pair-feeding, total parenteral nutrition, and everted intestinal sacs have been performed to elucidate the mechanisms behind the wasting syndrome (U.S. EPA, 1984, 1985; WHO/IPCS,

DRAFT--DO NOT QUOTE OR CITE

1989), but no single explanation has been obtained so far. No generalized impairment of intestinal absorption seems to occur.

Peterson et al. (1984) have suggested a model for the TCDD-induced wasting syndrome that is based on the assumption that body weight in rats is regulated to an internal standard or hypothalamically programmed set-point. Thus, body weight at a given age is constantly being compared to this set-point value, and if differences occur, feed consumption is adjusted. When TCDD lowers this set point, reduction in food consumption results as the rat attempts to reduce its weight to a new lower level. This hypothesis has been tested in several experiments under carefully controlled feeding conditions. Repeated studies have demonstrated that reduction of feed intake due to increased food spillage is sufficient to account for the loss of body weight in TCDD-treated SD rats. Additionally, TCDD-treated rats maintain and defend their reduced weight level with the same precision that *ad libitum*-fed control rats defend their normal weight level (Seefeld and Peterson, 1983, 1984; Seefeld et al., 1984a, b); the percentage of the daily feed intake that is absorbed by the gastrointestinal tract of TCDD-treated and control rats is similar (Potter et al., 1986; Seefeld and Peterson, 1984). Hypophagia was the major cause of adipose and lean tissue loss in male Fischer 344 rats, C57Bl/6 mice, and albino guinea pigs when exposed to a calculated LD₅₀ dose of TCDD. Body weight loss followed a similar time-course in TCDD-treated and pair-fed control animals of all three species (Kelling et al., 1985).

Thus, body weight loss appears to contribute to lethality in a species- and strain-dependent fashion, but weight loss appears to play a greater role in causing death in SD rats and guinea pigs than it does in Fischer 344 rats and C57Bl/6 mice. Loss of body weight and loss of appetite are also prominent signs of thyroid dysfunction. However, some data indicate that the effect of TCDD on thyroid hormones cannot explain the TCDD-induced decrease in body weight gain.

TCDD-induced wasting is always accompanied by the loss of adipose tissue. The rate of fat storage is determined by Lipoprotein lipase (LPL), which controls the serum level of triglycerides. Brewster and Matsumura (1984) found that the LPL activity was decreased in

DRAFT--DO NOT QUOTE OR CITE

guinea pigs to 20 percent of the value of *ad libitum*-fed controls after 1 day, and this effect persisted throughout the study (10 days). Thus, the authors suggest that TCDD irreversibly reduces adipose LPL activity, thus making the animals less capable of adapting to nutritional changes and needs.

In a series of studies on Wistar rats, Lakshman et al. (1988, 1989, 1991) have demonstrated that single intraperitoneal injections of TCDD (from 1 $\mu\text{g}/\text{kg}$) caused a dose-dependent inhibition of fatty acid synthesis in the liver and the adipose tissue. The adipose tissue was found to be more sensitive than the liver. Furthermore, they found an increased mobilization of depot fat into the plasma compartment accompanied by an increase in plasma free fatty acid concentrations.

In vitro studies in isolated heart mitochondria have indicated that a TCDD concentration of 1.5 nmol/mg mitochondrial protein affects oxygen activation associated with cell respiration. Superoxide radicals and H_2O_2 were indicated to be involved in the development of the observed effects (Nohl et al., 1989).

Loss of muscle tissue accompanied by a decreased glucocorticoid receptor-binding capacity and an increased glutamine synthetase activity have been observed in male Fischer 344N rats given a single oral TCDD dose of 100 $\mu\text{g}/\text{kg}$ (Max and Silbergeld, 1987).

Another biochemical effect associated with TCDD-induced wasting syndrome is the decrease in hepatic vitamin A storage in TCDD-exposed animals (Thunberg et al., 1979; Håkansson et al., 1989b, 1991). Vitamin A is necessary for growth, and vitamin A deficiency will result in depressed body weight gain as well as in reduced food intake. However, in contrast to TCDD-treated animals, the vitamin A-deficient animals continue to eat and grow, though body weight gain is less than normal (Hayes, 1971).

That decreased feed intake could be a result of a direct TCDD effect on the brain was initially indicated by Pohjanvirta et al. (1989), but this has been contradicted by later studies (Stahl and Rozman, 1990). The administration of TCDD at 50 $\mu\text{g}/\text{kg}$ intraperitoneally to male SD rats caused a significant decrease in the serum concentration of prolactin detectable after 4 hours, compared to pair-fed vehicle controls and noninjected controls (Jones et al.,

1987). The rapid onset of this effect suggests that it may be mediated by a pathway other than through interaction with the Ah receptor. Further studies have demonstrated that the effect of TCDD was reversed by pimozide, a dopamine receptor antagonist, and that the rate constant of dopamine depletion after α -methyl-*p*-tyrosine, as well as the turnover rate, were significantly elevated. This suggests a hypothalamic site of action of TCDD in their experiments (Russell et al., 1988).

Changes in intermediary metabolism have been demonstrated in TCDD-treated experimental animals. Conflicting data on effects on serum glucose and hepatic glucogen levels have been reported earlier (WHO/IPCS, 1989). Several recent studies have suggested that the ultimate cause of death in some mammalian species may be caused by a progressive hypoglycemia (Ebner et al., 1988; Gorski and Rozman, 1987; Gorski et al., 1990). However, in the guinea pig, serum glucose levels were not affected by treatment of the animals with TCDD (Gasiewicz and Neal, 1979). Slight reductions in serum glucose levels were noted in both Long-Evans and Han/Wistar rats (Pohjanvirta et al., 1989). Rozman et al. (1990) have suggested that the subchronic and chronic toxicities of TCDD are related to the inhibition of key enzymes of gluconeogenesis. They demonstrated that the induction of appetite suppression was preceded by the inhibition of PEPCK, which caused a reduction in gluconeogenesis. This was followed by a progressive increase in plasma tryptophan levels that was suggested to cause a serotonin-mediated reduction of the feed intake. In SD rats, TCDD in doses of 25 and 125 μ g caused a rapid decrease (50 percent) in phosphoenolpyruvate carboxykinase (PEPCK) activity 2 days after dosing, followed by a dose-dependent decrease in glucose-6-phosphatase activity 4 or 8 days after exposure. Both appetite suppression and reduced PEPCK activity occurred in the same dose range (Weber et al., 1991). TCDD-induced impairments of carbohydrate synthesis have also been suggested by studies in chick embryos (Lentnek et al., 1991).

Numerous studies have measured serum levels of free fatty acids, cholesterol, and triglycerides in various species after TCDD treatment (WHO/IPCS, 1989), but no pronounced qualitative differences have been observed between species or strains of mice.

The wasting syndrome thus seems to be a generalized effect, elicited in all species and strains, but at various dosages (single or repeated administration). Specific studies have not been performed to elucidate if this syndrome is elicited through the interaction of TCDD with the Ah receptor. However, strong support for an Ah receptor-mediated mechanism comes from studies with other CDDs and CDFs. The binding affinities of various CDDs and CDFs to the Ah receptor as well as those of related PCBs have been shown to strongly correlate with their potency of induction of the wasting syndrome in both rats and guinea pigs (Safe, 1990).

3.5.2. Hepatotoxicity

TCDD induces hyperplasia and hypertrophy of parenchymal cells and, thus, hepatomegaly in all species investigated, even at sublethal doses. There is, however, considerable variation in the extent and severity of this lesion among the species tested. Other liver lesions are more species specific. Lethality following the administration of TCDD cannot be explained by these liver lesions alone, although they may be a contributing factor, at least in the rat and rabbit. The morphological changes in the liver are accompanied by impaired liver function, which is characterized by liver enzyme leakage, increased microsomal monooxygenase activities, porphyria, impaired plasma membrane function, hyperlipidemia, and increased regenerative DNA synthesis (U.S. EPA, 1984, 1985; WHO/IPCS, 1989).

The hepatotoxic reaction in various strains of rats given lethal doses of TCDD is characterized by degenerative and necrotic changes, with the appearance of mononuclear cell infiltration, multinucleated giant hepatocytes, increased numbers of mitotic figures and pleomorphism of cord cells, an increase in the hepatic smooth endoplasmatic reticulum, and parenchymal cell necrosis. The histological findings are accompanied by hyperbilirubinemia, hypercholesterolemia, hyperproteinemia, and increased SGOT and SGPT activities, further indicating damaged liver function (WHO/IPCS, 1989). These lesions may be severe enough

DRAFT--DO NOT QUOTE OR CITE

to be a contributing factor in death. The lesions observed after sublethal doses are qualitatively almost identical to those observed after lethal doses.

Earlier studies in mice have found similar effects. Recently, Shen et al. (1991) reported a comparative study on the hepatotoxicity of TCDD in Ah-responsive and Ah-nonresponsive mice (C57BL/6J and DBA/2J, respectively). C57BL/6J mice given a single dose of 3 $\mu\text{g/kg}$ TCDD developed mild to moderate hepatic lipid accumulation but no inflammation or necrosis. Severe fatty change, mild inflammation, and necrosis occurred at 150 $\mu\text{g/kg}$. DBA/2J mice given 30 $\mu\text{g/kg}$ developed hepatocellular necrosis and inflammation but no fatty change. Lipid accumulation was only slight after 600 $\mu\text{g/kg}$. The authors concluded that the Ah locus may be involved in determining the steatotic effects of TCDD.

The guinea pig shows less severe morphological alteration in the liver than other species. Likewise, the hamster exhibits little or no liver damage even after a fatal dose, but liver lesions have been observed after prolonged periods following the administration of nonlethal doses.

Several parameters relating to disturbed hepatic plasma membranae function have been studied (U.S. EPA, 1984, 1985; WHO/IPCS, 1989). ATPase activities were depressed and protein kinase C activity was increased in rats, but not in guinea pigs, treated with TCDD (Bombick et al., 1985). TCDD also induced a decrease in the binding of EGF. The relative doses of TCDD needed to suppress EGF binding to 50 percent of the control level were 1, 14, and 32 $\mu\text{g/kg}$ for the guinea pig, the SD rat, and the Syrian Golden hamster, respectively (Madhukar et al., 1984). A single intraperitoneal dose of 115 $\mu\text{g TCDD/kg bw}$ decreased the EGF binding by 93.1, 97.8, and 46.0 percent in C57BL/6, CBA, and AKR mice, respectively, 10 days after treatment (Madhukar et al., 1984).

Further studies on the interaction of TCDD with EGF have been performed in congeneric mice of the strain C57BL/6J (Lin et al., 1991a, b). The ED_{50} for the TCDD-induced decrease in maximum binding capacity of the EGF receptor was 10 times higher in the Ah-nonresponsive mice, compared to the Ah-responsive animals. This study supports the

DRAFT--DO NOT QUOTE OR CITE

hypothesis that the effects of TCDD on EGF receptor ligand binding may be mediated by the Ah receptor.

The effects of TCDD on biliary excretion of various compounds have also been studied. Of special interest are studies on the excretion of ouabain, a model compound for neutral nonmetabolized substrates such as estradiol, progesterone, and cortisol, which was depressed in a dose-related manner by a single, oral dose of TCDD in rats (Yang et al., 1977, 1983b). The available data suggest that the hepatic membrane transport of ouabain may be selectively impaired by TCDD. Peterson et al. (1979a, b) have indicated that changes in ATPase activities are not responsible for the reduced ouabain excretion.

TCDD administration stimulates the accumulation of porphyrins in the liver and an increase in urinary porphyrin excretion. Indeed, during manifest porphyria, accumulation of porphyrins occurs not only in the liver but also in the kidney and spleen of rats (Goldstein et al., 1982).

Contradictory results on species variations have been published. It seems clear that porphyria can be produced in both mice and rats but the condition is always the result of subchronic or chronic administration. Exposure to single doses has not been demonstrated to produce porphyria. The mechanism underlying the induction of porphyria is not elucidated. Cantoni et al. (1981) exposed rats orally to 0.01, 0.1, and 1 μg TCDD/kg bw/week for 45 weeks and increased coproporphyrin levels were observed at all dose levels. A marked porphyric state appeared only at the highest dose tested, after 8 months of exposure.

TCDD is a potent inducer of rodent and murine ALA-synthetase, the initial and rate-limiting enzyme involved in heme synthesis. However, increased ALA activity was not found in mice exposed to 25 μg TCDD/kg bw/week for 11 weeks, despite porphyria being evident (Jones and Sweeney, 1980). Thus, the induction of ALA-synthetase does not seem to be a necessary event in TCDD-induced porphyria. A more likely suggestion is that decreased hepatic porphyrinogen decarboxylase is the primary event in porphyria induced by halogenated aromatics (Elder et al., 1976, 1978). TCDD depresses this enzyme activity in

vivo in the liver of mice (Cantoni et al., 1984a, b; Elder and Sheppard, 1982; Jones and Sweeney, 1980), but not in vitro (Cantoni et al., 1984b).

A comparative study of TCDD-induced porphyria has not been conducted in responsive and nonresponsive mice. However, in a study on Ah-responsive (Ah^b) and Ah-nonresponsive (Ah^d) C57BL/6J female mice, the urinary excretion of porphyrins was examined after treatment of the animals with hexachlorobenzene for ≤ 17 weeks (Hahn et al., 1988). After 15 weeks of treatment with 200 ppm hexachlorobenzene in the diet, the excretion of porphyrins was 200 times higher in the Ah^b mice, compared with controls. In contrast, the Ah^d mice only showed a sixfold increase. Induction of P-450c(1A1) was observed only in Ah^b mice, while induction of P-450d(1A2) was observed in both strains, but to a lesser degree in the Ah^d mice.

3.5.3. Epidermal Effects

Chloracne and associated dermatological changes are widespread responses to TCDD in humans. However, this type of toxicity is expressed only in a limited number of animal species (i.e., rabbits, monkeys, and hairless mice).

In the rabbit ear bioassay, a total dose of 80 ng TCDD gave a chloracnegenic response, while no response was obtained when the total dose applied to the ear was 8 ng (Jones and Krizek, 1962; Schwetz et al., 1973). The application of TCDD in various vehicles has been demonstrated to markedly decrease this response (Poiger and Schlatter, 1980). The hairless mouse is a less sensitive model for chloracnegenic response than is the rabbit ear bioassay (Knutson and Poland, 1982; Puhvel et al., 1982). However, following repeated applications of $\sim 0.1 \mu\text{g}$ TCDD over several weeks, an acnegenic response was noted in the hairless mouse strains, SkH:HR1 and HRS/J. An acnegenic response was also caused by repeated applications of 2 mg of 3,4,3',4'-TCB (Puhvel et al., 1982). Female HRS/J hairless mice have also been used to test the dermal toxicity and skin tumor-promoting activity of TCDD, PeCDF, and HxCDF (Hebert et al., 1990a). All of the tested

DRAFT--DO NOT QUOTE OR CITE

compounds induced coarse, thickened skin with occasional desquamation; these effects were more severe after the application of PeCDF and HxCDF.

Keratinocytes, the principal cell type in the epidermis, have been utilized as an *in vitro* model for studies of TCDD-induced hyperkeratosis both in human- and animal-derived cell cultures. The response to TCDD is analogous to the hyperkeratinization observed *in vivo*.

A TCDD-induced keratinization response *in vitro* was first demonstrated in a keratinocyte cell line derived from a mouse teratoma (XB cells). The keratinization was dose-related (Knutson and Poland, 1980b). Late-passage XB cells (termed XBF cells) lost their ability to respond by keratinization after TCDD treatment. Both XB cells (keratinization assay) and XBF cells (flat-cell assay) have proven to be useful in *in vitro* bioassays to determine the dioxinlike activities of both environmental samples and pure isomers (Gierthy and Crane, 1985a, b; Gierthy et al., 1984).

Several continuous lines of human keratinocytes, derived from neonatal foreskin or squamous cell carcinomas, have been shown to respond to TCDD in nM concentrations with a variety of signs indicating alterations in the normal differentiation process (WHO/IPCS, 1989). The responses include decreased DNA synthesis, decreased number of proliferating basal cells, decreased binding of EGF, and an increase in the state of differentiation (Osborne and Greenlee, 1985; Hudson et al., 1986). The responses were also obtained with TCDF, but not with 2,4-diCDD (Osborne and Greenlee, 1985). TCDD has also been shown to inhibit high-density growth arrest in human squamous carcinoma cell lines, and, indeed, the minimum concentration for increases in cell proliferation was 0.1 nM in the most sensitive cell line (SCC-15G). In studies on the same cell lines, a modulating effect of the transforming growth factor beta could not be demonstrated (Hebert et al., 1990 b, c).

3.5.4. Enzyme Induction

TCDD has repeatedly been found to increase the activities of various enzymes. While observations of enzyme inhibition have also been made, enzyme induction has been one of

DRAFT--DO NOT QUOTE OR CITE

the most extensively studied biochemical responses produced by TCDD. The mixed-function oxidase (MFO) system is the most thoroughly investigated, and AHH and EROD (as markers for CYP1A1 induction) are the most frequently assayed enzyme activities. The induction of MFO activities might potentiate the toxicity of other foreign compounds that require metabolic transformation by the MFO system before they can exert their toxic effects. Furthermore, increased MFO activities might adversely affect important metabolic conversions of endogenous compounds. TCDD has also been reported to affect a variety of other enzymes (e.g., UDPGT and GST) that are components of multifunctional enzyme systems involved in the conjugation, biotransformation, and detoxification of a wide variety of endogenous and exogenous compounds.

Several investigators have studied the relative potency of various halogenated dioxins, dibenzofurans, and biphenyls to induce AHH and/or EROD activities (Safe, 1990). An apparent structure-activity relationship was found between the location of the halogen atoms on the dibenzo-*p*-dioxin molecule and the ability to induce AHH activity both in vivo and in vitro. Isomers with halogens at the four lateral ring positions produced a greater biological response than those with halogens at three lateral ring positions, while two lateral halogen atoms seemed to be insufficient to produce a biological response. Numerous studies have indicated that there is very strong agreement between the Ah-binding affinity of various CDDs, CDFs, and related PCBs and their potency to induce AHH, both in vivo and in vitro (Safe, 1990). Structure-activity studies have also demonstrated clear correlation between the toxicity and induction potency of a series of CDDs, CDFs, and coplanar PCBs (Poland and Glover, 1973; Safe, 1990).

On a molecular basis, TCDD is the most potent MFO-inducing compound known, and MFO induction seems to be the most sensitive biochemical response produced. Measurements of the induction of AHH or EROD (mediated through CYP1A1) are considered to be very sensitive markers of the TCDD-induced enzyme induction. According to Kitchin and Woods (1979), induction in the rat takes place at doses as low as 0.002 μg TCDD/kg bw. The NOEL for a single administration to rats seems to be 1 ng/kg, while a

DRAFT--DO NOT QUOTE OR CITE

single dose of 3 ng/kg causes a detectable induction of AHH or EROD (Kitchin and Woods, 1979; Abraham et al., 1988).

Enzyme induction has also been observed in the offspring of various species after prenatal and postnatal (milk) exposure to TCDD (Lucier et al., 1975; Korte et al., 1990; Wærn et al., 1991b).

The effect of TCDD on enzyme activities has been most frequently investigated in the rat (WHO/IPCS, 1989). In the liver, TCDD has been shown to increase both the contents of cytochrome P-450IA1 and cytochrome P-450IA2, as well as other microsomal enzyme activities involved in the oxidative transformation and conjugation of xenobiotics (e.g., aniline hydroxylase, AHH, biphenyl hydroxylase, ECOD, EROD, and UDPGT) (U.S. EPA, 1984, 1985; WHO/IPCS 1989).

TCDD also affects some other hepatic enzymes not related to the MFO system, including aldehyde dehydrogenase, β -aminolevulinic acid synthetase, DT-diaphorase, transglutaminase, ornithine decarboxylase, transaminases (ALT and AST), plasma membrane ATPases, porphyrinogen carboxylase, prostaglandin synthetase, enzymes involved in testosterone metabolism, and RNA polymerase (U.S. EPA, 1984, 1985; WHO/IPCS, 1989).

Studies in different species also have revealed that enzyme induction due to TCDD exposure is both a species- and strain-specific phenomenon. Pohjanvirta et al. (1988) studied enzyme induction in the Long-Evans and Han/Wistar (Kuopio) rat strains (LD_{50} , ~10 and >3,000 μ g/kg, respectively). Differences in the inducibility of EROD, ECOD, or ethylmorphine N-demethylase were not found, nor were there any differences with regard to the amount of available Ah receptor or the amount of cytochrome P-450 in the hepatic microsomal fractions. Similarly, differences regarding possible induction of UDPGT were absent (Pohjanvirta et al., 1990).

Enzyme induction studies on mice have been performed mainly with strains that are genetically different at the Ah locus, thus making them responsive or nonresponsive to the induction of hepatic cytochrome P-450IA1-related enzyme activities. Qualitatively and in general, the same responses can be obtained in both strains, but there may be more than one

DRAFT--DO NOT QUOTE OR CITE

order of magnitude difference with regard to the doses required to elicit a response. TCDD is thus 10-fold more potent in inducing hepatic cytochrome P-450IA1 and the related AHH activity in C57BL/6J mice (Ah-responsive) than in DBA/2 mice (Ah-nonresponsive) (Poland and Knutson, 1982; Nebert, 1989).

In contrast, although the guinea pig is the most sensitive species to the toxic effects of TCDD, it does not respond to the administration of TCDD with liver toxicity or with extensive enzyme induction. Indeed, even at lethal doses, the induction of MFO is only very slight (Beatty and Neal, 1977; Håkansson et al., 1992).

The data on enzyme induction in rabbits are rather limited and also somewhat conflicting with regard to increases in the amount of cytochrome P-450 (Hook et al., 1975; Liem et al., 1980).

Similarly, hepatic enzyme induction has been only partially studied in Syrian Golden hamsters. When hamsters were given a lethal dose of TCDD, increased hepatic GST and glutathione reductase activities were found. The ED₅₀ values for the induction of hepatic ECOD and reduced NADP:menadione oxidoreductase activities and cytochrome P-450 content in male Syrian Golden hamsters were 1.0, 2.0, and 0.5 µg TCDD/kg bw, respectively (i.e., extremely low doses, compared to doses that produce tissue damage and lethality in this species) (Gasiewicz et al., 1986).

In a comparative study of EROD induction in guinea pigs, rats, C57BL/6 and DBA/2 mice, and Syrian Golden hamsters, the animals were given single doses that were intended to be equitoxic (i.e., 1, 40, 100, 400, and 400 µg TCDD/kg, respectively) compared with the acute toxicity for the respective species and strain. EROD induction was noted in all species except for the hamster. During the observation period (112 days), the EROD induction dropped to more or less normal values in all rats and mice, while the induction (albeit low compared with the other species) was sustained for the whole period in the guinea pig (Håkansson et al., 1992).

The N-demethylation of caffeine has been applied as a noninvasive method for studying enzyme induction in vivo. Studies on the marmoset monkey (*Callithrix jacchus*)

DRAFT--DO NOT QUOTE OR CITE

utilizing ^{14}C -labeled caffeine and measuring $^{14}\text{CO}_2$ exhalation by a breath test has indicated a NOEL of 1 ng/kg and a LOEL of 3 ng/kg (Kruger et al., 1990). Although the authors state that the N-demethylation of caffeine probably was P-450IA1 dependent, studies by Butler et al. (1989) indicate that this reaction is dependent on cytochrome P-450IA2.

In the chick embryo, both AHH and δ -aminolevulinic acid synthetase have been reported to be extremely sensitive to the inductive effects of TCDD and related compounds (Poland and Glover, 1973; Brunström and Andersson, 1988; Brunström, 1990).

Although TCDD is relatively nontoxic in cell cultures, it is a very potent inducer of AHH or EROD activities in in vitro systems, including lymphocytes and primary hepatocytes, as well as established and transformed cell lines.

The ED_{50} values for AHH induction by TCDD have been determined in 11 established cell lines and in fetal primary cultures from five animal species and cultured human lymphocytes. The values ranged from 0.04 ng/mL medium in C57Bl/6 mouse fetal cultures and 0.08 ng/mL in the rat hepatoma H-4-II-E cell line to >66 ng/mL in the HTC rat hepatoma cell line (Niwa et al., 1975). Several cultured human cells or cell lines including lymphocytes (Atlas et al., 1976), squamous cell carcinoma lines (Hudson et al., 1983), breast carcinoma cell lines (Jaiswal et al., 1985), and lymphoblastoid cells (Nagayama et al., 1985) have been shown to be inducible for AHH activity by TCDD.

TCDD was demonstrated to be the most potent AHH inducer of 24 chlorinated dibenzo-*p*-dioxin analogues (Bradlaw et al., 1980) in a rat hepatoma cell culture (H-4-II-E) that is extremely sensitive to AHH induction. The EC_{50} values for AHH and EROD induction in the same cell system varied over 7 orders of magnitude for 14 different CDDs, the most potent being TCDD and the least potent being 2,3,6-triCDD (Mason et al., 1986).

3.5.5.1. Appraisal

Based on data from Kitchin and Woods (1979), Abraham et al. (1988), and Kruger et al. (1990), Neubert (1991) has calculated NOEL values for enzyme induction in both rats and marmoset monkeys to a single dose of 1 ng/kg bw. At this dose, the tissue concentrations

for both species were found to be 4 ppt for adipose tissue and 3 ppt for the liver. It is interesting to note that the wide range of sensitivities toward the acute toxicity of TCDD is also reflected in a wide range of sensitivities for enzyme induction both *in vivo* and *in vitro*. However, it is evident that the guinea pig is fairly insensitive to enzyme induction, while the hamster is highly sensitive in this respect.

Finally, it is evident that the structure-activity relationships revealed from *in vitro* testing correlate fairly well with *in vivo* studies within a given species or strain.

3.5.6. Endocrine Effects

Alterations in endocrine regulation have been suggested from human exposure to TCDD that resulted in hirsutism and chloracne. Chronic exposure to TCDD causes impaired reproduction in experimental animals, possibly by interfering with the estrus cycle in combination with some steroidlike activities of TCDD. This has prompted studies on the interaction of TCDD with steroid hormones and their receptors.

Increased systemic levels of glucocorticoids may mimic some of the symptoms of TCDD toxicity (e.g., involution of lymphoid tissues, edema, and mobilization of fatty acids from adipose tissues). Thus, TCDD has been suggested to increase glucocorticoid activity through indirect effects on glucocorticoid receptors. Poland et al. (1976) demonstrated that cortisol and synthetic glucocorticoids did not bind to the TCDD receptor.

Conflicting data have been reported on TCDD-induced levels of glucocorticoids. However, significant changes to the liver cytosolic glucocorticoid receptor were induced by TCDD at doses 10,000-fold lower in adrenalectomized SD rats, compared with control rats (Sunahara et al., 1989). The data furthermore indicate that it is the binding properties of the receptor that are affected rather than the amount of receptor protein. Studies in congeneric strains of Ah-responsive and Ah-nonresponsive C57BL/6J female mice (Goldstein et al., 1990; Lin et al., 1991a, b) have also demonstrated that TCDD decreased the maximum binding capacity of the hepatic glucocorticoid receptor in both strains of mice by ~30 percent. Differences in dose-response curves between the different strains could not be

observed. These data suggest that this effect may be mediated by a pathway different from that mediated by the Ah receptor.

Steroids are endogenous substrates for the hepatic MFO system. TCDD, which influences the activity of this enzyme system, may thus alter steroid metabolism *in vivo* and, consequently, also the magnitude of steroid-mediated functions.

Early studies also reported contradictory data on changes in steroid levels. However, Umbreit and Gallo (1988) suggest that estrogen receptor modulation and the animal's physiological response to this modulation can explain some of the toxicity observed in TCDD-treated animals. The susceptibility of different species to TCDD correlates, to some extent, with their steroid glucuronidation capacity. Thus, hamsters have low steroid UDPGT activity while guinea pigs have a corresponding high activity. Another example is given by comparing the SD and Gunn rat, the latter being defective in producing some UDPGTs. The homozygous Gunn rat is 3-10 times more resistant to the effects of TCDD than is the SD rat (Thunberg, 1984; Thunberg and Håkansson, 1983). However, the results of TCDD exposure in various species and strains are complex. [In order to counteract the TCDD-induced modulation of the estrogen receptor, the effects observed will be dependent on the ability of the organism to synthesize and excrete estrogens.] Interactions of TCDD and related compounds with estrogen have recently been reviewed by Safe et al. (1991).

The importance of estrogens as modulators of TCDD-induced toxicity has also been demonstrated by Lucier et al. (1991), who found that the tumor-promoting effects of TCDD could be effectively prevented by removing the ovaries from female rats before exposure to TCDD. This finding agrees well with the results obtained from the long-term bioassays that demonstrated liver tumors only in female rats (Kociba et al., 1978; NTP, 1982).

In studies on congenic strains of Ah-responsive and Ah-nonresponsive C57BL/6J female mice, a statistically significant difference in the responsiveness of the hepatic estrogen receptor was found, thus indicating that the Ah receptor regulates the effects of TCDD on the binding of estrogen to the hepatic estrogen receptor (Goldstein et al., 1990; Lin et al., 1991a, b).

DRAFT--DO NOT QUOTE OR CITE

TCDD-induced changes in levels or activities of testosterone or its metabolites have been reported from several studies (Keys et al., 1985; Mittler et al., 1984; Moore and Peterson, 1985; Neal et al., 1979). A single oral dose of 50 μg TCDD/kg bw increased the plasma corticosterone level in SD rats 7 and 14 days postexposure (Neal et al., 1979). However, a single oral dose of 25 μg TCDD/kg bw has also been shown to decrease the plasma corticosterone in SD rats 14 and 21 days postexposure. It is essential to note that Neal et al. (1979) also observed a slight decrease in serum corticosterone during days 1-4 posttreatment.

Mittler et al. (1984) demonstrated a decreased activity of testicular 16- α -testosterone hydroxylase, 6- β -hydroxytestosterone, and 7- α -hydroxytestosterone in young SD rats 90 hours postexposure to single intraperitoneal doses of 0.2, 1, or 5 μg TCDD/kg bw.

A single dose of 0.06 μmol TCDD/kg bw decreased levels of 3 α -, 6 α -, and 16 β -hydroxytestosterone; an increase of 7 α -hydroxytestosterone have also been observed in young male Wistar rats (Keys et al., 1985). Moore et al. (1985) also noted decreases in serum testosterone and dihydrotestosterone levels in 15 μg TCDD/kg bw-dosed male SD rats. The data do not, however, allow for any conclusions with regard to the possible relationship to receptor-mediated toxicity. TCDD induces several enzymes related to testosterone metabolism, which suggests that the changes observed may be secondary to the induction of various enzymes. Serum testosterone and dihydrotestosterone were found to be dose-dependently depressed by TCDD treatment in male SD rats, when compared with pair-fed and *ad libitum*-fed controls. The ED_{50} for this effect was ~ 15 $\mu\text{g}/\text{kg}$ (Moore et al., 1985). It was further shown that testosterone synthesis was decreased in the animals due to depressed production of pregnenolone by the testis (Kleeman et al., 1990). In the same strain of rats, a single oral dose of TCDD of 100 $\mu\text{g}/\text{kg}$ was found to cause a 55 percent decrease in testicular cytochrome P-450_{xx} activity but also to cause the inhibition of the mobilization of cholesterol to cytochrome P-450_{xx}. The authors concluded that the latter effect probably was responsible for the inhibition of testicular steroidogenesis (Moore et al., 1991). Maternal exposure to TCDD has been shown to affect the male reproductive system

at low doses (i.e., the lowest dose tested was 64 ng/kg) (Mably et al., 1991, 1992a, b, c) (see Chapter 5).

In ovo exposure of white Leghorn chickens to TCDD in the dose range of 1-10,000 pmol/egg increased the cardiac release of prostaglandins (Quilley and Rifkind, 1986). Studies on chick embryos have indicated that the induction of cytochrome P-450 by TCDD results in a major increase in the NADPH-dependent metabolism of arachidonic acid (Rifkind et al., 1990). These effects are thus clearly related to receptor-mediated enzyme induction.

Rather conflicting data have been published regarding TCDD-induced effects on thyroid hormones (WHO/IPCS, 1989). The available data on serum T4, T3, and TSH levels are not sufficient to state whether or not TCDD-treated rats are functionally hypothyroidic, euthyroidic, or hyperthyroidic.

However, Brouwer (1987) has demonstrated that a dioxinlike PCB (i.e., 3,4,3',4'-TCB, through a rapidly produced metabolite, 5-OH-TCB) binds to TTR. This binding causes interactions with the physiological functions of TTR and thyroid hormone transport is severely affected. This finding may explain some of the characteristic toxicological lesions found after PCB exposure.

3.5.7. Vitamin A Storage

Decreased hepatic vitamin A storage has been reported in animals exposed to various chlorinated aromatic compounds. Because minute quantities are needed to produce its effect and its persistence in nature TCDD is unique in its ability to reduce the vitamin A content of the liver. A single oral dose of 10 µg TCDD/kg bw decreased both the total amount and the concentration of vitamin A in the liver of adult male SD rats (Thunberg et al., 1979). The decrease was evident 4 days after dosing and progressed with time. After 8 weeks, the treated animals had a total liver vitamin A content corresponding to 33 percent of that of controls. Decreased dietary intake of vitamin A could not account for this difference. A significant increase in the UDPGT activity was observed, suggesting an increased excretion of glucuronide-conjugated vitamin A. However, no correlation between the UDPGT activity

and the hepatic vitamin A reduction was seen when homozygous Gunn rats lacking inducible UDPGT (Aitio et al., 1979) and heterozygous Gunn rats with inducible UDPGT were treated with a single, oral dose of 10 μ g TCDD/kg bw (Thunberg and Håkansson, 1983).

In a study combining pair-feed restriction and a single TCDD treatment, it was found that the decreases in liver reserves of vitamin A were not related to a decreased intake of vitamin A via the diet (Håkansson et al., 1989a).

Puhvel et al. (1991) reported a comparative study in which congenic haired (+/+) and hairless (hr/hr) HRS/J mice were fed a vitamin A-deficient diet and treated topically with TCDD. The sensitivity to TCDD-induced cutaneous changes was essentially 100 times higher in hairless mice than in haired mice (0.01 and 1.0 μ g 3 times/week for 3 and 2 weeks, respectively). In the haired phenotype, the effects of vitamin A depletion by itself were not seen by cutaneous histology, nor were any changes in cutaneous morphology attributable to TCDD observed. In the hairless mice, however, vitamin A deficiency increased the keratinization of dermal epithelial cysts and increased the sensitivity of these cysts to TCDD-induced keratinization. Analysis of vitamin A demonstrated that TCDD-exposure did not affect cutaneous levels of the vitamin but did significantly lower levels of vitamin A in the liver. TCDD-induced body weight loss and atrophy of the thymus glands were not affected by the vitamin A status in either strain.

In a study on tumor promotion by TCDD, utilizing the induction of enzyme-altered hepatic foci in the liver and performed on female SD rats, Flodström et al. (1991) found that vitamin A deficiency by itself enhanced foci development. The effect of TCDD treatment was also markedly enhanced, as were other TCDD-induced toxicities including thymus atrophy.

Several studies have been performed to elucidate the mechanism of TCDD-vitamin A interaction. Håkansson et al. (1989c) and Håkansson and Hanberg (1989) have demonstrated that TCDD specifically inhibits the storage of vitamin A in liver stellate cells. Brouwer et al. (1989) demonstrated that a single dose of TCDD (10 μ g/kg) to female SD rats reduced vitamin A in the liver, lung, intestines, and adrenal glands, while increasing its concentration

in serum, kidneys, and urine. They also found a 150 percent increase in the free fraction of serum retinol binding protein. Taken together, all of these data in the rat indicate that TCDD induces an increased mobilization of vitamin A from hepatic and extrahepatic storage sites into the serum, accompanied by an enhanced elimination of the vitamin via the kidney into the urine.

In a comparative study of TCDD toxicity in male SD rats and Hartley guinea pigs (Håkansson et al., 1989b), the animals were given single intraperitoneal doses of 40 and 0.5 $\mu\text{g/kg}$ bw, respectively (i.e., comparable fractions of their respective LD_{50}). In these species there were similar reductions in hepatic vitamin A, while serum and renal vitamin A concentrations were increased in the rat but unaffected in the guinea pig. Hepatic EROD activity was markedly increased in the rat but unchanged in the guinea pig. Furthermore, although rats seemed to recover from the wasting, thymic atrophy, and liver enlargement, and resumed their ability to store vitamin A in the liver at 4-8 weeks after exposure, no such trends for wasting and vitamin A storage were observed in guinea pigs, even 16 weeks after exposure. A complementary study also included C57BL/6 mice, DBA/2 mice, and Syrian Golden hamsters (Håkansson et al., 1991). The effects on TCDD-induced decrease of vitamin A in the liver and the lung correlated reasonably well with other toxic symptoms observed in the animals. On the other hand, studies on two strains of rats, Long-Evans and Han/Wistar (the Han/Wistar being > 300 times more resistant to TCDD toxicity) could not demonstrate significant differences in the TCDD-induced changes in vitamin A in the liver, kidney, testicles, or serum after a sublethal dose (4 $\mu\text{g/kg}$) (Pohjanvirta et al., 1990). These findings show that the correlations between TCDD-induced lethality and changes in vitamin A status found among other species also apply to these strains of rats.

The interaction of 3,4,3',4'-TCB with vitamin A has been studied by Brouwer and Van den Berg (1983, 1984, 1986), Brouwer et al. (1985, 1986a, b), and Brouwer (1987). The effects of TCB on vitamin A differ in many respects from those of TCDD. TCB is rapidly converted in vivo into a polar 5-OH-TCB metabolite, which binds with a relatively high affinity to TTR. As a consequence of this interaction, the physiological functions of

TTR in retinoid and thyroid hormone transport are severely affected in TCB-exposed animals. The model proposed by Brouwer (1987) may explain some of the characteristic toxicological lesions related to PCB exposure. This mechanism of action seems to be clearly separated from the Ah receptor-mediated toxicity of CDDs and CDFs. Hydroxylated metabolites of TCDD have also been demonstrated to bind in a similar manner to TTR (Lans et al., 1992). However, due to the very slow metabolism of TCDD (or other 2,3,7,8-substituted CDDs/CDFs), this mechanism of toxicity probably plays a very minor role in the toxicity.

Taken together, these data indicate that TCDD interferes with the storage mechanism for vitamin A. Because supplementation of dietary vitamin A seems to be unable to counteract all of the observed toxic effects, this would imply either that the effect on vitamin A storage is secondary to TCDD toxicity or that the cellular utilization of vitamin A is affected by TCDD.

3.5.8. Lipid Peroxidation

Lipid peroxidation and oxidative stress have been indicated as factors that affect the acute toxicity of TCDD (WHO/IPCS, 1989; Wahba et al., 1989a, b, 1990a, b; Pohjnavirta et al., 1989; Alsharif et al., 1990; Stohs et al., 1990). Among the effects noted have been membrane lipid peroxidation, decreased membrane fluidity, and increased incidence of single-strand breaks in DNA. Studies relating these observations to the Ah receptor have not been performed. However, when considering the available data on TCDD and lipid peroxidation, it is not possible to attempt to define a relationship between lipid peroxidation and TCDD-induced lethality.

3.6. MECHANISMS OF TOXICITY

Despite extensive research to elucidate the ultimate event(s) underlying the toxic action of TCDD, definite information is not yet available. The toxicity of TCDD apparently depends on the fact that the four lateral positions of the molecule are occupied by chlorine. Toxicity decreases with decreasing lateral substitution and increasing total chlorine substitution. TCDD toxicity involves many different types of symptoms; these symptoms vary from species to species and from tissue to tissue, both quantitatively and qualitatively. Furthermore, age- and sex-related differences in sensitivity have been reported. Another characteristic of TCDD toxicity is the delay before all the endpoints of toxicity are manifested (from 2 weeks to 2 months), which is seen in all species.

Polymorphism in the Ah locus, which has been suggested to be the structural gene for the cytosolic receptor, seems to determine the sensitivity of genetically different strains of mice to TCDD and congeners. Ah-responsive strains of mice (e.g., C57Bl/6) are characterized by high hepatic levels of the TCDD-receptor protein, highly elevated levels of hepatic cytochrome P-4501A1 and associated enzyme activities in response to treatment with 3-MC (3-methylcholanthrene), and sensitivity to the ulcerative action of DMBA (7,12-dimethylbenz(a)anthracene) on the skin. Ah-nonresponsive mice (e.g., DBA/2) lack these characteristics.

Based on these findings, several genetic studies have been performed to elucidate the role of the receptor in TCDD toxicity. In contrast to 3-MC, TCDD induces AHH activity and several toxic effects both in Ah-responsive and Ah-nonresponsive strains of mice. However, the dose required to produce the effect in an Ah-nonresponsive strain is approximately 10-fold greater than that needed in an Ah-responsive strain. This indicates that the Ah-nonresponsive strain also contains the TCDD receptor but this receptor is defective (Okey and Vella, 1982). Data from studies of DBA/2 mice given either single or multiple doses of TCDD (Jones and Sweeney, 1980; Smith et al., 1981) suggest that the LD₅₀ in this strain of mice is at least fivefold greater than the values recorded for the C57Bl/6 and C57Bl/10 strains (Jones and Greig, 1975; Smith et al., 1981; Vos et al., 1974). TCDD-induced

DRAFT--DO NOT QUOTE OR CITE

hepatic porphyria has also been shown to segregate with the Ah locus in mice (Jones and Sweeney, 1980). The correlative differences between the C57Bl/6 and DBA/2 strains of mice, in terms of altered specific binding of TCDD and sensitivity to this compound, may be unique and may not be applicable to other species (Gasiewicz and Rucci, 1984).

In a genetic-crossing experiment between Long-Evans and Han/Wistar rats (Pohjanvirta, 1990), it was demonstrated that the F₁ offspring were as resistant to TCDD toxicity as the Han/Wistar rats (LD₅₀ > 3,000 µg/kg). Further studies on the F₂ generation indicated that the distribution of resistant and susceptible phenotypes was consistent with inheritance regulated by two (possibly three) autosomal genes displaying complete dominance, independent segregation, and an additive coefficient. Thus, in contrast to the findings in mice, TCDD resistance seems to be a dominant trait in the rat.

Less convincing evidence for the model of a receptor-mediated toxicity of TCDD arises from studies of the toxicity, receptor levels, and/or enzyme induction of TCDD in various species, tissues, and cell cultures. Despite enormous variability in the recorded LD₅₀ values for the guinea pig, rat, mouse, rabbit, and hamster, the amounts and physical properties of the hepatic as well as extrahepatic receptors are comparable in these species (Gasiewicz and Rucci, 1984; Poland and Knutson, 1982). Furthermore, although the recorded LD₅₀ values for TCDD vary > 100 times between the chick embryo, the C3H/HeN mouse, and the SD rat, the ED₅₀ doses for AHH induction in these species are comparable (Poland and Glover, 1974). Even between strains of rats with a difference of > 300 times in LD₅₀, no differences in enzyme induction could be demonstrated (Pohjanvirta et al., 1988). In the guinea pig, the most TCDD-susceptible species, AHH induction is not a prominent symptom, even at lethal doses of TCDD. A number of cell types, including primary cultures and established and transformed cell lines from several species and tissues, are inducible for AHH activity, indicating the presence of the receptor, yet toxicity is not expressed in these systems (Knutson and Poland, 1980a). The available data thus suggest that the receptor for TCDD may be a prerequisite but is not sufficient in itself for the mediation of toxicity.

TCDD toxicity mimics in many respects endocrine imbalance, although evidence indicating a direct involvement of hormones in the toxic action of TCDD does not exist. However, the studies by Lucier et al. (1991) clearly indicate the importance of interactions with estrogen regulation.

The most reliable and consistent symptom of TCDD toxicity among all experimental animals is that of weight loss. The cause of the body weight loss seems to be reduced food intake apparently occurring secondarily to a physiological adjustment that reduces the body weight to a maintenance level that is lower than normal. The physiological trigger for this body weight set-point might be a target for TCDD.

The ability of TCDD to impair vitamin A storage may be responsible for some of the toxic effects produced by TCDD.

3.7. CONCLUSIONS

From the complex picture that evolves from the above outlined data, it is amply evident that TCDD elicits a plethora of toxic responses, both after short-term and long-term exposure. The lowest doses (single or repeated) that have been demonstrated to elicit various biological responses in certain animals have been compiled in Table 3-4. The analysis of the various signs and symptoms that occur in various species and strains may lead to the following conclusions:

- When comparing species and strains, it is clearly evident that there are enormous differences in the sensitivity to specific TCDD-induced toxicities. This conclusion is valid for almost all the responses studied. Qualitatively, however, there seems to be fairly good agreement between the types of responses that can be observed (i.e., almost all responses can be produced in every species and strain if the right dose is chosen). In highly sensitive species (e.g., the guinea pig), lethality may prevent a response from occurring.
- Our present knowledge rules out enzyme induction, as such, as being the cause of toxicity and death. Although the toxicokinetics of TCDD vary between species, these differences are not sufficient to explain the variabilities in

TABLE 3-4

Lowest Effect Levels for Biological Responses of 2,3,7,8-TCDD in Experimental Animals

Species	Dose or concentration and duration	Effect	Reference
Guinea pigs	2.0 µg/kg-single oral dose	acute lethality (single dose LD ₅₀)	McConnell et al., 1978a McNulty, 1977
Rhesus monkey	1.0 µg/kg-single oral dose	acute (systemic) toxicity	McNulty, 1977
Sprague-Dawley rat	2.0 ng/kg-single oral dose ^a	induction of AHH (CYP1A1)	Kitchin and Woods, 1979
Marmoset monkey	3.0 ng/kg-single oral dose	induction of N-demethylation (CYP1A2)	Kruger et al., 1990
Guinea pig	1 ng/kg-day for 8 weeks	immunosuppression (decreased response to tetanus toxin)	Zinkl et al., 1973
C57B/6 mouse	1 ng/kg-week for 4 weeks intraperitoneally	immunosuppression (decreased generation of CTL)	Clark et al., 1983
Rhesus monkey	500 ppt in diet for 9 months (12 ng/kg-day); 2 ppb in diet for 61 days (50 ng/kg-day)	chronic lethality	Allen et al., 1977; McNulty, 1977
Rhesus monkey	50 ppt in diet for 20 months (1.5 ng/kg-day)	chronic toxicity (hair loss)	Schantz et al., 1978
Sprague-Dawley rat	10 ng/kg-day for 2 years in feed	porphyrin metabolism	Kociba et al., 1978

^a0.6 ng/kg = no effect level

DRAFT--DO NOT QUOTE OR CITE

sensitivity to TCDD toxicity (see Chapter 1). The available data indicate an involvement of TCDD in processes regulating cellular differentiation and/or division as well as those controlling estrogen homeostasis. Alterations in the regulation of such processes, which are not equally active in all cells throughout the organism, would be expected to result in effects that vary among tissues as well as among species.

- The overwhelming number of toxic responses to TCDD (including lethality) typically show a delay in their appearance, which supports the assumption that these responses are not the result of a direct insult from the compound.
- The induction of hepatic cytochrome P-450-dependent monooxygenases (mainly CYP1A1) is one of the hallmarks of TCDD exposure. This effect has been demonstrated to be mediated through the interaction with a specific protein called the Ah receptor. This process covers binding of TCDD to the receptor, followed by binding of the receptor-ligand complex to DNA recognition sites leading to expression of specific genes and translation of their protein products, which then mediate their biological effects.
- Studies in congenic mice that are Ah responsive or Ah nonresponsive have demonstrated that the majority of TCDD-induced toxic responses segregate quantitatively with the Ah locus. However, the amount of Ah receptor expressed in most laboratory species and strains is rather comparable. The Ah receptor is thus unlikely to be the only determinant of TCDD-induced toxicity. Rather, it has to be assumed that the species and strain differences are confined to the latter parts of the receptor-mediated chain of events (i.e., binding of the receptor-ligand complex to DNA and the subsequent expression of specific genes). Another explanation may be that the binding affinity of the Ah receptor is different or defective. In addition, some of the responses may be secondary in the sense that they are caused by the altered homeostasis of endogenous compounds caused by the TCDD-induced increased activities of various enzymes.
- It has repeatedly been reported as the current opinion that all known effects of TCDD are probably Ah receptor mediated (e.g., Roberts, 1991). However, except for the chain of events leading to the induction of certain enzymes, clear evidence for such a conclusion is still lacking. Nevertheless, the studies in congenic mice in combination with the usually rather strong correlation between enzyme induction and various other TCDD-induced toxic responses make the assumption rather likely. Further support for the probability of a

DRAFT--DO NOT QUOTE OR CITE

receptor-mediated process is provided by the very strong structure-activity relationships that have been demonstrated between various CDDs/CDFs and a variety of toxic responses.

3.8. REFERENCES

- Abraham, K.; Krowke, R.; Heubert, D. (1988) Pharmacokinetics and biological activity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. 1. Dose-dependent tissue distribution and induction of hepatic ethoxyresorufin O-deethylase in rats following a single injection. Arch. Toxicol. 62:359-368.
- Ahlborg, U.G.; Håkansson, H.; Lindström, G.; Rappe, C. (1990) Studies on the retention of individual polychlorinated dibenzofurans (PCDFs) in the liver of different species. Chemosphere 20:1235-1240.
- Aitio, A.; Parkki, M.G.; Marniemi, J. (1979) Different effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on glucuronide conjugation of various aglycones. Studies in Wistar and Gunn rats. Toxicol. Appl. Pharmacol. 47:55-60.
- Allen, J.R.; Lalich, J.J. (1962) Response of chickens to prolonged feeding of crude "toxic fat." Proc. Soc. Exp. Biol. Med. 109:48-51.
- Allen, J.R.; Barsotti, D.A.; Van Miller, J.P.; Abrahamson, L.; Lalich, J.J. (1977) Morphological changes in monkeys consuming a diet containing low levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Food Cosmet. Toxicol. 15(5):401-410.
- Alsharif, N.Z.; Grandjean, C.J.; Murray, W.J. (1990) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)-induced decrease in the fluidity of rat liver membranes. Xenobiotica 20(9): 979-988.
- Astroff, B.; Zacharewski, T.; Safe, S.; Arlotto, M.P.; Parkinson, A.; Thomas, P.; Levine, W. (1988) 6-Methyl-1,3,8-trichlorodibenzofuran as a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin antagonist: inhibition of the induction of rat cytochrome P450 isozymes and related monooxygenase activities. Mol. Pharmacol. 33:231-236.
- Atlas, S.A.; Vesell, E.S.; Nebert, D.W. (1976) Genetic control of interindividual variations in the inducibility of aryl hydrocarbon hydroxylase in cultured human lymphocytes. Cancer Res. 36:4619-4630.
- Barsotti, D.A.; Abrahamson, L.J.; Allen, J.R. (1979) Hormonal alterations in female rhesus monkeys fed a diet containing 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Bull. Environ. Contam. Toxicol. 21:463-469.
- Beatty, P.; Neal, R.A. (1977) Factors affecting the induction of D-5-diaphorase by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Biochem. Pharmacol. 27:505.

DRAFT--DO NOT QUOTE OR CITE

- Beatty, P.W.; Vaughn, W.K.; Neal, R.A. (1978) Effect of alteration of rat hepatic mixed-function oxidase (MFO) activity on the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Toxicol. Appl. Pharmacol.* 45:513-519.
- Beatty, P.W.; Lembach, K.J.; Holscher, M.A.; Neal, R.A. (1975) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on mammalian cells in tissue cultures. *Toxicol. Appl. Pharmacol.* 31:309-312.
- Birnbaum, L.S.; McDonald, M.M.; Blair, P.C.; Clark, A.M.; Harris, M.W. (1990) Differential toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in C57BL/6J mice congenic at the Ah locus. *Fund. Appl. Toxicol.* 15:186-200.
- Bombick, D.W.; Madhukar, B.V.; Brewster, D.W.; Matsumura, F. (1985) TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) causes increases in protein kinases particularly protein kinase C in the hepatic plasma membrane of the rat and the guinea pig. *Biochem. Biophys. Res. Commun.* 127(1):296-302.
- Bradlaw, J.A.; Garthoff, L.H.; Hurley, N.E.; Firestone, D. (1980) Comparative induction of aryl hydrocarbon hydroxylase activity in vitro by analogues of dibenzo-*p*-dioxin. *Food. Cosmet. Toxicol.* 18:627-635.
- Brewster, D.W.; Matsumura, F. (1984) TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) reduces lipoprotein lipase activity in the adipose tissue of the guinea pig. *Biochem. Biophys. Res. Commun.* 122:810-816.
- Brewster, D.W.; Matsumura, F.; Akera, T. (1987) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the guinea pig heart muscle. *Toxicol. Appl. Pharmacol.* 89:408-417.
- Brewster, D.W.; Bombick, D.W.; Matsumura, F. (1988) Rabbit serum hypertriglyceridemia after administration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *J. Toxicol. Environ. Health* 26:495-507.
- Brouwer, A. (1987) Interference of 3,4,3',4'-tetrachlorobiphenyl in vitamin A (retinoids) metabolism: possible implications for toxicity and carcinogenicity of polyhalogenated aromatic hydrocarbons. Thesis.
- Brouwer, A.; Van den Berg, K.J. (1983) Early decrease in retinoid levels in mice after exposure to low doses of polychlorinated biphenyls. *Chemosphere* 12:555-557.

DRAFT--DO NOT QUOTE OR CITE

- Brouwer, A.; Van den Berg, K.J. (1984) Early and differential decrease in natural retinoid levels in C57BL/Rij and DBA/2 mice by 3,4,3',4'-tetrachlorobiphenyl. *Toxicol. Appl. Pharmacol.* 73:204-209.
- Brouwer, A.; Van den Berg, K.J. (1986) Binding of a metabolite of 3,4,3',4'-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex, carrying both retinol and thyroxine. *Toxicol. Appl. Pharmacol.* 85:301-312.
- Brouwer, A.; Van den Berg, K.J.; Kukler, A. (1985) Time and dose responses of the reduction in retinoid concentrations in C57BL/Rij and DBA/2 mice induced by 3,4,3',4'-tetrachlorobiphenyl. *Toxicol. Appl. Pharmacol.* 78:180-189.
- Brouwer, A.; Van den Berg, K.J.; Blaner, W.S.; Goodman, D.S. (1986a) Transthyretin (prealbumin) binding of PCBs, a model for the mechanism of interference with vitamin A and thyroid hormone metabolism. *Chemosphere* 15:1699-1706.
- Brouwer, A.; Blaner, W.S.; Kukler, A.; Van den Berg, K.J. (1986b) Interference of 3,4,3',4'-tetrachlorobiphenyl with the plasma transport protein complex of vitamin A in rodents leading to a marked reduction in serum retinol and retinol binding protein levels. *Toxicol. Appl. Pharmacol.* (Unpublished)
- Brouwer, A.; Håkansson, H.; Kukler, A.; Van den Berg, K.J. (1989) Marked alterations in retinoid homeostasis of SD rats induced by a single i.p. dose of 10 µg/kg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicology* 56:267-283.
- Brunström, B. (1990) Mono-*ortho*-chlorinated chlorobiphenyls: toxicity and induction of 7-ethoxyresorufin O-deethylase (EROD) activity in chick embryos. *Arch. Toxicol.* 64:188-192.
- Brunström, B.; Andersson, L. (1988) Toxicity and 7-ethoxyresorufin O-deethylase-inducing potency of coplanar polychlorinated biphenyls (PCBs) in chick embryos. *Arch. Toxicol.* 62:263-266.
- Butler, M.A.; Iwasaki, M.; Guengerich, F.P.; Kadubar, F.F. (1989) Human cytochrome P-450_{PA} (P1501A2, the phenacetin O-deethylase) is primarily responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic arylamines. *Proc. Natl. Acad. Sci.* 86:7696-7700.

DRAFT--DO NOT QUOTE OR CITE

- Canga, L.; Levi, R.; Rifkind, A.B. (1988) Heart as a target organ in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity: decreased β -adrenergic responsiveness and evidence of increased intracellular calcium. *Proc. Natl. Acad. Sci.* 85:905-909.
- Cantoni, L.; Salmona, M.; Rizzardini, M. (1981) Porphyrinogenic effect of chronic treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in female rats. Dose-effect relationship following urinary excretion of porphyrins. *Toxicol. Appl. Pharmacol.* 57:156-163.
- Cantoni, L.; Dal Fiume, D.; Ferraroli, A.; Salmona, M.; Ruggieri, R. (1984a) Different susceptibility of mouse tissues to porphyrogenic effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Lett.* 20:201-210.
- Cantoni, L.; Dal Fiume, D.; Rizzardini, M.; Ruggieri, R. (1984b) In vitro inhibitory effect on porphyrinogen carboxylase of liver extracts from TCDD-treated mice. *Toxicol. Lett.* 20:211-217.
- Chapman, D.E.; Schiller, C.M. (1985) Dose-related effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in C57BL/6J and DBA/2J mice. *Toxicol. Appl. Pharmacol.* 78:147-157.
- Chastain, J.E.; Pazdernik, T.L. (1985) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)-induced immunotoxicity. *Int. J. Immunopharmacol.* 7(6):849-856.
- Clark, D.A.; Sweeney, G.; Safe, S.; Hancock, E.; Kilburn, D.G.; Gauldie, J. (1983) Cellular and genetic basis for suppression of cytotoxic T-cell generation by haloaromatic hydrocarbons. *Immunopharmacology* 6:143-153.
- DeCaprio, A.P.; McMartin, D.N.; O'Keefe, P.W.; Rej, R.; Silkworth, J.B.; Kaminsky, L.S. (1986) Subchronic oral toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the guinea pig: comparisons with a PCB-containing transformer fluid pyrolysate. *Fundam. Appl. Toxicol.* 6:454-463.
- Ebner, K.; Brewster, D.W.; Matsumura, F. (1988) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on serum insulin and glucose levels in the rat. *J. Environ. Sci. Health.* B23:427-438.
- Elder, G.H.; Sheppard, D.M. (1982) Immunoreactive uroporphyrinogen decarboxylase is unchanged in porphyria caused by TCDD and hexachlorobenzene. *Biochem. Biophys. Res. Comm.* 109:113-120.

DRAFT--DO NOT QUOTE OR CITE

- Elder, G.H.; Evans, J.O.; Matlin, S.A. (1976) The effect of porphyrinogenic compound, hexachlorobenzene, on the activity of hepatic uroporphyrinogen decarboxylase in the rat. Clin. Sci. Mol. Med. 51:71-80.
- Elder, D.G.; Lee, G.B.; Tovey, J.A. (1978) Decreased activity of hepatic uroporphyrinogen decarboxylase in sporadic porphyria cutanea tarda. N. Engl. J. Med. 299:274-278.
- Firestone, D. (1973) Etiology of chick edema disease. Environ. Health Perspect. 5:59-66.
- Flodström, S.; Busk, L.; Kronevi, T.; Ahlborg, U.G. (1991) Modulation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and phenobarbital-induced promotion of hepatocarcinogenesis in rats by the type of diet and vitamin A deficiency. Fundam. Appl. Toxicol. 16:375-391.
- Gasiewicz, T.A.; Neal, R.A. (1979) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin tissue distribution, excretion, and effects on clinical chemical parameters in guinea pigs. Toxicol. Appl. Pharmacol. 51:329-339.
- Gasiewicz, T.A.; Rucci, G. (1984) Cytosolic receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Evidence for a homologous nature among various mammalian species. Mol. Pharmacol. 26:90-98.
- Gasiewicz, T.A.; Rucci, G.; Henry, E.C.; Baggs, R.B. (1986) Changes in hamster hepatic cytochrome P-450, ethoxycoumarin *o*-deethylase, and reduced NAD(P): menadione oxidoreductase following treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Partial dissociation of temporal and dose-response relationships from elicited toxicity. Biochem. Pharmacol. 35:2737-2742.
- Geyer, H.J.; Scheunert, I.; Rapp, K.; Kettrup, P.; Korte, F.; Greim, H.; Rozman, K. (1990) Correlation between acute toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and total body fat content in mammals. Toxicology 65:97-107.
- Gierthy, J.F.; Crane, D. (1985a) In vitro bioassay for dioxinlike activity based on alterations in epithelial cell proliferation and morphology. Fundam. Appl. Toxicol. 5:754-759.
- Gierthy, J.F.; Crane, D. (1985b) Development of in vitro bioassays for chlorinated dioxins and dibenzofurans. In: Chlorinated dioxins and dibenzofurans in the total environment II. Keith, L.H.; Rappe, C.; Choudhury, G., eds. Boston, MA: Butterworth Publishers.

DRAFT--DO NOT QUOTE OR CITE

- Gierthy, J.F.; Crane, D.; Frenkel, G.D. (1984) Application of an in vitro keratinization assay to extracts of soot from a fire in a polychlorinated biphenyl-containing transformer. *Fundam. Appl. Toxicol.* 4:1036-1041.
- Goldstein, J.A.; Linko, P.; Bergman, H. (1982) Induction of porphyria in the rat by chronic versus acute exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Biochem. Pharmacol.* 31:1607-1613.
- Goldstein, J.A.; Lin, F.H.; Stohs, S.J.; Graham, M.; Clarke, G.; Birnbaum, L.; Lucier, G. (1990) The effects of TCDD on receptors for epidermal growth factor, glucocorticoid, and estrogen in Ah-responsive and -nonresponsive congenic mice and the effects of TCDD on estradiol metabolism in a liver tumor promotion model in female rats. In: *Mouse liver carcinogenesis: mechanisms and species comparisons*. Alan R. Liss, Inc. pp. 187-202.
- Gorski, J.R.; Rozman, K. (1987) Dose-response and time course of hypothyroxinemia and hypoinsulinemia and characterization of insulin hypersensitivity in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-treated rats. *Toxicology* 44:297-307.
- Gorski, J.R.; Weber, L.W.D.; Rozman, K. (1990) Reduced gluconeogenesis in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-treated rats. *Arch. Toxicol.* 64:66-71.
- Göttlicher, M.; Cikryt, P.; Wiebel, F.J. (1990) Inhibition of growth by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in 5L rat hepatoma cells is associated with the presence of Ah receptor. *Carcinogenesis* 11(12):2205-2210.
- Greig, J.B.; Jones, G.; Butler, W.H.; Barnes, J.M. (1973) Toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Food Cosmet. Toxicol.* 11:585-595.
- Hahn, M.E.; Gasiewicz, T.A.; Linko, P.; Goldstein, J.A. (1988) The role of the Ah locus in hexachlorobenzene-induced porphyria: studies in the congenic C57BL/6J mice. *Biochem. J.* 254:245-254.
- Håkansson, H.; Hanberg, A. (1989) The distribution of [¹⁴C]-2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and its effect on vitamin A content in parenchymal and stellate cells of rat liver. *J. Nutr.* 119:573-580.
- Håkansson, H.; Johansson, L.; Ahlborg, U.G.; Moore, R.W.; Peterson, R.E. (1989a) Hepatic vitamin A storage in relation to paired feed restriction and TCDD-treatment. *Chemosphere* 19(1-6):919-920.

DRAFT--DO NOT QUOTE OR CITE

- Håkansson, H.; Johansson, L.; Manzoor, E.; Ahlborg, U.G. (1989b) 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD)-induced alterations in the vitamin A homeostasis and in the 7-ethoxyresorufin O-deethylase (EROD)-activity in SD rats and Hartley guinea pigs. *Chemosphere* 18(1-6):299-305.
- Håkansson, H.; Hanberg, H.; Ahlborg, U.G. (1989c) The distribution of [¹⁴C]2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) between parenchymal and non-parenchymal rat hepatic cells and its effect on the vitamin A content of these cells. *Chemosphere* 18:307-312.
- Håkansson, H.; Ahlborg, U.G.; Johansson, L.; Poiger, H. (1990) Vitamin A storage in rats subchronically exposed to PCDDs/PCDFs. *Chemosphere* 20(7-9):1147-1159.
- Håkansson H.; Johansson, L.; Manzoor, E.; Ahlborg, U.G. (1991) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the vitamin A status of Hartley guinea pigs, SD rats, C57Bl/6 mice, DBA/2 mice, and Golden Syrian hamsters. *J. Nutr. Sci. Vitaminol.* 37:117-138.
- Håkansson, H.; Johansson, L.; Manzoor, E.; Ahlborg, U.G. (1992) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on 7-ethoxyresorufin-*O*-deethylase activity in Hartley guinea pigs, SD rats, C57Bl/6 mice, DBA/2 mice, and Golden Syrian hamsters. (Manuscript in preparation.)
- Hanberg, A.; Ståhlberg, M.; Georgellis, A.; de Wit, C.; Ahlborg, U.G. (1991) Swedish dioxin survey: evaluation of the H-4-II E bioassay for screening environmental samples for dioxin-like enzyme induction. *Pharmacol. Toxicol.* 69(6):442-449.
- Harris, M.W.; Moore, J.A.; Vos, J.G.; Gupta, B.N. (1973) General biological effects of TCDD in laboratory animals. *Environ. Health Perspect. Exp.* 5:101-109.
- Hayes, K.C. (1971) On the pathophysiology of vitamin A deficiency. *Nutr. Rev.* 29:3-6.
- Hebert, C.D.; Harris, M.W.; Elwell, M.R.; Birnbaum, L.S. (1990a) Relative toxicity and tumor-promoting ability of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PCDF) and 1,2,3,4,7,8-hexachlorodibenzofuran (HCDF). *Toxicol. Appl. Pharmacol.* 102:362-377.
- Hebert, C.D.; Cao, L.; Birnbaum, L.S. (1990b) Inhibition of high-density growth arrest in human squamous carcinoma cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Carcinogenesis* 11:1335-1342.

DRAFT--DO NOT QUOTE OR CITE

- Hebert, C.D.; Cao, Q.L.; Birnbaum, L.S. (1990c) Role of transforming growth factor beta in the proliferative effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on human squamous carcinoma cells. *Cancer Res.* 50:7190-7197.
- Henck, J.M.; New, M.A.; Kociba, R.J.; Rao, K.S. (1981) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: acute oral toxicity in hamsters. *Toxicol. Appl. Pharmacol.* 59:405-407.
- Hochstein, J.R.; Aulierich, R.J.; Bursian, S.J. (1988) Acute toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to mink. *Arch. Environ. Contam. Toxicol.* 17:23-27.
- Hook, G.E.R.; Haseman, J.K.; Lucier, G.W. (1975) Induction and suppression of hepatic and extrahepatic microsomal foreign-compound-metabolizing enzyme systems by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Chem.-Biol. Interact.* 10:199.
- Hudson, L.G.; Shaikh, R.; Toscano, W.A.; Greenlee, W.F. (1983) Induction of 7-ethoxycoumarin-*O*-deethylase activity in cultured human epithelial cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Evidence for TCDD receptor. *Biochem. Biophys. Res. Commun.* 115:611-617.
- Hudson, L.G.; Toscano, W.A., Jr.; Greenlee, W.F. (1985) Regulation of epidermal growth factor binding in a human keratinocyte cell line by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* 77:251-259.
- Hudson, L.G.; Toscano, W.A.; Greenlee, W.F. (1986) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) modulates epidermal growth factor (EGF) binding to basal cells from a human keratinocyte cell line. *Toxicol. Appl. Pharmacol.* 82:481-492.
- Jaiswal, A.K.; Nebert, D.W.; Eilsen, H.W. (1985) Comparison of aryl hydrocarbon hydroxylase and acetanilide 4-hydroxylase induction by polycyclic aromatic compounds in human and mouse cell lines. *Biochem. Pharmacol.* 34:2721-2731.
- Jones, E.L.; Krizek, H. (1962) A technique for testing acnegenic potency in rabbits, applied to the potent acnegen, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Invest. Dermatol.* 39:511-517.
- Jones, G.; Greig, J.B. (1975) Pathological changes in the liver of mice given 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Experientia* 31:1315-1317.
- Jones, K.G.; Sweeney, G.D. (1980) Dependence of the porphyrogenic effect of 2,3,7,8-tetrachlorodibenzo(*p*)dioxin upon inheritance of aryl hydrocarbon hydroxylase responsiveness. *Toxicol. Appl. Pharmacol.* 53:42-49.

DRAFT--DO NOT QUOTE OR CITE

- Kawamoto, T.; Matsumura, F.; Madhukar, B.V.; Bombick, D.W. (1989) Effects of TCDD on the EGF receptor of XB mouse keratinizing epithelial cells. *J. Biochem. Toxicol.* 4:173-182.
- Kelling, C.K.; Christian, B.J.; Inhorn, S.L.; Peterson, R.E. (1985) Hypophagia-induced weight loss in mice, rats, and guinea pigs treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Fundam. Appl. Toxicol.* 5:700-712.
- Kelling, C.K.; Menahan, L.A.; Peterson, R.E. (1987) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin treatment on mechanical function of the rat heart. *Toxicol. Appl. Pharmacol.* 91:497-501.
- Keys, B.; Hlavinka, M.; Mason, G.; Safe, S. (1985) Modulation of rat hepatic microsomal testosterone hydroxylases by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related toxic isostereomers. *Can. J. Pharmacol.* 63:1537-1542.
- Kimmig, J.; Schultz, K.H. (1957) Chlorierte aromatische zyklische äther als ursache der sogenannten chlorakne. *Dermatologica*. [Basel] 115(4):540-546.
- Kitchin, K.T.; Woods, J.S. (1979) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) effects on hepatic microsomal cytochrome P-448-mediated enzyme activities. *Toxicol. Appl. Pharmacol.* 47:537-546.
- Kleeman, J.M.; Moore, R.W.; Peterson, R.E. (1990) Inhibition of testicular steroidogenesis in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated rats: evidence that the key lesion occurs prior to or during pregnenolone formation. *Toxicol. Appl. Pharmacol.* 106:112-125.
- Knutson, J.C.; Poland, A. (1980a) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: failure to demonstrate toxicity in twenty-three cultured cell types. *Toxicol. Appl. Pharmacol.* 54:377-383.
- Knutson, J.C.; Poland, A. (1980b) Keratinization of mouse teratoma cell line XB produced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: an in vitro model of toxicity. *Cell* 22:27-36.
- Knutson, J.C.; Poland, A. (1982) Response of murine epidermis to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: interaction of the Ah and hr loci. *Cell* 30:225-234.
- Kociba, R.J.; Keeler, P.A.; Park, C.N.; Gehring, P.J. (1976) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD): results of a 13-week oral toxicity study in rats. *Toxicol. Appl. Pharmacol.* 35:553-574.

DRAFT--DO NOT QUOTE OR CITE

- Kociba, R.J.; Keyes, D.G.; Beyer, J.E.; Carreon, R.M.; Wade, C.E.; Dittenber, D.A.; Kalnins, R.P.; Frauson, L.E.; Park, C.N. (1978) Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in rats. *Toxicol. Appl. Pharmacol.* 46:279-303.
- Kociba, R.J.; Keyes, D.G.; Beyer, J.E.; Carreon, R.M.; Gehring, P. (1979) Long-term toxicologic studies of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in laboratory animals. *Ann. N.Y. Acad. Sci.* 320:397-404.
- Korte, M.; Stahlmann, R.; Neubert, D. (1990) Induction of hepatic monooxygenases in female rats and offspring in correlation with TCDD tissue concentrations after single treatment during pregnancy. *Chemosphere* 20:1193-1198.
- Kruger, N.; Neubert, B.; Helge, H.; Neubert, D. (1990) Induction of caffeine-demethylations by 2,3,7,8-TCDD in marmoset monkeys measured with a ¹⁴CO₂-breath test. *Chemosphere* 20:1173-1176.
- Lakshman, M.R.; Campbell, B.S.; Chirtel, S.J.; Ekarohita, N. (1988) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on *de novo* fatty acid and cholesterol synthesis in the rat. *Lipids* 23(9):904-906.
- Lakshman, M.R.; Chirtel, S.J.; Chambers, L.L.; Coutlakis, P.J. (1989) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on lipid synthesis and lipogenic enzymes in the rat. *J. Pharmacol. Exp. Ther.* 248(1):62-66.
- Lakshman, M.R.; Ghosh, P.; Chirtel, S.J. (1991) Mechanism of action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on intermediary metabolism in the rat. *J. Pharmacol. Exp. Ther.* 258(1):317-319.
- Lans, M.C.; Klasson-Wheeler, E.; Willemsen, M.; Meussen, E.; Safe, S.; Brouwer, A. (1992) Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, -polychlorodibenzo-*p*-dioxins and -polychlorodibenzofurans with human transthyrethrin. *Chem.-Biol. Interact.* (Manuscript in preparation)
- Lentnek, M.; Griffith, O.W.; Rifkind, A.B. (1991) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin increases reliance on fats as a fuel source independently of diet: evidence that diminished carbohydrate supply contributes to dioxin lethality. *Biochem. Biophys. Res. Commun.* 174(3):1267-1271.

DRAFT--DO NOT QUOTE OR CITE

- Liem, H.H.; Muller-Eberhard, U.; Johnson, E.F. (1980) Differential induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin of multiple forms of rabbit microsomal cytochrome P-450: evidence for tissue specificity. *Mol. Pharmacol.* 18:565.
- Lin, F.H.; Stohs, S.J.; Birnbaum, L.S.; Clark, G.; Lucier, G.W.; Goldstein, J.A. (1991a) The effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the hepatic estrogen and glucocorticoid receptors in congenic strains of Ah-responsive and Ah-nonresponsive C57BL/6J mice. *Toxicol. Appl. Pharmacol.* 108:129-139.
- Lin, F.H.; Clark, G.; Birnbaum, L.S.; Lucier, G.W.; Goldstein, J.A. (1991b) Influence of the Ah locus on the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the hepatic epidermal growth factor receptor. *Mol. Pharmacol.* 39:307-313.
- Lucier, G.W.; Sonawane, B.R.; McDaniel, O.S.; Hook, G.E.R. (1975) Postnatal stimulation of hepatic microsomal enzymes following administration of TCDD to pregnant rats. *Chem.-Biol. Interact.* 11:15-26.
- Lucier, G.W.; Tritscher, A.; Goldsworthy, J.; Foley, J.; Clark, G.; Goldstein, J.; Maronpot, R. (1991) Ovarian hormones enhance 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated increases in cell proliferation and preneoplastic foci in a two-stage model for rat hepatocarcinogenesis. *Cancer Res.* 51:1391-1397.
- Luster, M.I.; Boorman, G.A.; Dean, J.H.; Harris, M.W.; Luebke, R.W.; Padarathsingh, M.L.; Moore, J.A. (1980) Examination of bone marrow, immunologic parameters and host susceptibility following pre- and postnatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Intl. J. Immunopharmacol.* 2:301-310.
- Luster, M.I.; Hong, L.H.; Boorman, G.A.; Clark, G.; Hayes, H.T.; Greenlee, W.F.; Dold, K.; Tucker, A.N. (1985) Acute myelotoxic responses in mice exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Toxicol. Appl. Pharmacol.* 81:156-165.
- Mably, T.A.; Moore, R.W.; Bjerke, D.L.; Peterson, R.E. (1991) The male reproduction system is highly sensitive to in utero and lactational TCDD exposure. In: Banbury report: biological basis for risk assessment of dioxins and related compounds, Vol. 35. Gallo, M.A.; Scheuplein, J.; van der Heijden, K.A., eds. Cold Spring Harbor Laboratory. Plainview, NY. pp. 69-78.
- Mably, T.A.; Moore, R.W.; Goy, R.W.; Peterson, R.E. (1992a) In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: 1. Effects on androgenic status. *Toxicol. Appl. Pharmacol.* 114:97-107.

DRAFT--DO NOT QUOTE OR CITE

- Mably, T.A.; Moore, R.W.; Goy, R.W.; Peterson, R.E. (1992b). In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: 2. Effects on sexual behavior and the regulation of luteinizing hormone secretion in adulthood. *Toxicol. Appl. Pharmacol.* 114:108-117.
- Mably, T.A.; Bjerke, D.L.; Moore, R.W.; Gendron-Fitzpatrick, A.; Peterson, R.E. (1992c) In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: 3. Effects on spermatogenesis and reproductive capability. *Toxicol. Appl. Pharmacol.* 114:118-126.
- Madhukar, B.V.; Brewster, D.W.; Matsumura, F. (1984) Effects of in vivo-administered 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on receptor binding of epidermal growth factor in the hepatic plasma membrane of rat, guinea pig, mouse, and hamster. *Proc. Natl. Acad. Sci. USA* 81:7407-7411.
- Mason, G.; Farrell, K.; Keys, B.; Piskorska-Pliszczyńska, J.; Safe, L.; Safe, S. (1986) Polychlorinated dibenzo-*p*-dioxins: quantitative in vitro and in vivo structure-activity relationships. *Toxicology* 41:21-31.
- Max, S.R.; Silbergeld, E.K. (1987) Skeletal muscle glucocorticoid receptor and glutamine synthetase activity in the wasting syndrome in rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* 87:523-527.
- McConnell, E.E. (1980) Acute and chronic toxicity, carcinogenesis, reproduction, teratogenesis and mutagenesis in animals. In: Halogenated biphenyls, perphenyls, naphthalenes, dibenzodioxins, and related products. Kimbrough, R.D., ed. Amsterdam: Elsevier Science Publ. pp. 109-150.
- McConnell, E.E.; Moore, J.A.; Dalgard, D.W. (1978a) Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rhesus monkeys (*Macaca mulatta*) following a single oral dose. *Toxicol. Appl. Pharmacol.* 43:175-187.
- McConnell, E.E.; Moore, J.A.; Haseman, J.K.; Harris, M.W. (1978b) The comparative toxicity of chlorinated dibenzo-*p*-dioxins in mice and guinea pigs. *Toxicol. Appl. Pharmacol.* 44:335-356.
- McNulty, W.P. (1977) Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin for rhesus monkeys: brief report. *Bull. Environ. Contam. Toxicol.* 18:108-109.
- McNulty, W.P. (1984) Fetotoxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for rhesus macaques (*Macaca mulatta*). *Am. J. Primatol.* 6:41-47.

DRAFT--DO NOT QUOTE OR CITE

- Mittler, J.C.; Ertel, N.H.; Peng, R.X.; Yang, C.S.; Kiernan, T. (1984) Changes in testosterone hydroxylase activity in rat testis following administration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Ann. N.Y. Acad. Sci.* 438:645-648.
- Moore, R.W.; Peterson, R.E. (1985) Enhanced catabolism and elimination of androgens do not cause the androgenic deficiency in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated rats. *Fed. Proc.* 44:518.
- Moore, J.A.; McConnell, E.E.; Dalgard, D.W.; Harris, M.W. (1979) Comparative toxicity of three halogenated dibenzofurans in guinea pigs, mice, and rhesus monkeys. *Ann. N.Y. Acad. Sci.* 320:151-163.
- Moore, R.W.; Potter, C.L.; Theobald, H.M.; Robinson, J.A.; Peterson, R.E. (1985) Androgenic deficiency in male rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* 79:99-111.
- Moore, R.W., Jefcoate, C.R.; Peterson, R.E. (1991) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin inhibits steroidogenesis in the rat testis by inhibiting the mobilization of cholesterol to cytochrome P450₁. *Toxicol. Appl. Pharmacol.* 109:85-97.
- Nagayama, J.; Kiyohara, C.; Masuda, Y.; Kuratsune, M. (1985) Genetically mediated induction of aryl hydrocarbon hydroxylase activity in human lymphoblastoid cells by polychlorinated dibenzofuran isomers and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Arch. Toxicol.* 56:230-235.
- Neal, R.A.; Beatty, P.W.; Gasiewicz, T.A. (1979) Studies of the mechanisms of toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Ann. N.Y. Acad. Sci.* 320:204-213.
- Neal, R.A.; Olson, J.R.; Gasiewicz, T.A.; Geiger, L.E. (1982) The toxicokinetics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in mammalian systems. *Drug Metab. Rev.* 13:355-385.
- Nebert, D.W. (1989) The Ah locus: genetic differences in toxicity, cancer, mutation, and birth defects. *Crit. Rev. Toxicol.* 20:137-152.
- Neubert, D. (1991) Animal data on the toxicity of TCDD and special aspects of risk assessment. Presented at a WHO consultation of tolerable daily intake of PCDDs and PCDFs from food, Bilthoven, The Netherlands, 1990.

DRAFT--DO NOT QUOTE OR CITE

- Niwa, A.; Kumaki, K.; Nebert, D.W. (1975) Induction of aryl hydrocarbon-hydroxylase activity in various cell cultures by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Mol. Pharmacol.* 11:399-408.
- Nohl, H.; De Silva, D.; Summer, K.-H. (1989) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin induces oxygen activation associated with cell respiration. *Free Radic. Biol. Med.* 6:369-374.
- Norback, D.H.; Allen, J.R. (1973) Biological responses of the nonhuman primate, chicken, and rat to chlorinated dibenzo-*p*-dioxin ingestion. *Environ. Health Perspect.* 5:233-240.
- NTP (National Toxicology Program). (1982) Carcinogenesis bioassay of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (CAS No. 1746-01-6) in Osborne-Mendel rat and B6C3F1 mice (gavage study). NTP Tech. Rept. Ser. 109. DHHS, PHS, NIH, Research Triangle Park, NC.
- Okey, A.B.; Vella, L.M. (1982) Binding of 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to a common Ah receptor site in mouse and rat hepatic cytosols. *Eur. J. Biochem.* 127:39-47.
- Olson, J.R.; Holscher, M.A.; Neal, R.A. (1980) Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the Golden Syrian hamster. *Toxicol. Appl. Pharmacol.* 55:67-78.
- Osborne, R.; Greenlee, W.F. (1985) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) enhances terminal differentiation of cultured human epidermal cells. *Toxicol. Appl. Pharmacol.* 77:434-443.
- Peterson, R.E.; Hamada, N.; Yang, K.H.; Madhukar, B.V.; Matsumura, F. (1979a) Depression of adenosine triphosphatase activities in isolated liver surface membranes of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated rats: correlation with effects on ouabain biliary excretion and bile flow. *J. Pharmacol. Exp. Ther.* 210:275-282.
- Peterson, R.E.; Hamada, N.; Yang, K.H.; Madhukar, B.V.; Matsumura, F. (1979b). Reversal of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced depression of ouabain biliary excretion by pregnenolone-(16)7C-carbonitrile and spironolactone in isolated perfused rat livers. *Toxicol. Appl. Pharmacol.* 50:407-416.

DRAFT--DO NOT QUOTE OR CITE

- Peterson, R.E.; Seefeld, M.D.; Christian, B.J.; Potter, C.L.; Kelling, K.; Keeseey, R. (1984) The wasting syndrome in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity: basic features and their interpretation. In: Banbury report: biological mechanisms of dioxin action, Vol. 18. Poland, A; Kimbrough, R., eds. Cold Spring Harbor Laboratory. Plainview, NY. pp. 291-308.
- Plüess, N.; Poiger, H.; Hohbach, C.; Suter, M.; Schlatter, C. (1988a) Subchronic toxicity of some chlorinated dibenzofurans (PCDFs) and a mixture of PCDFs and chlorinated dibenzodioxins (PCDDs) in rats. *Chemosphere* 17:937-984.
- Plüess, N.; Poiger, H.; Hohbach, C.; Suter, M.; Schlatter, C. (1988b) Subchronic toxicity of 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) in rats. *Chemosphere* 17:1099-1110.
- Pohjanvirta, R. (1990) TCDD resistance is inherited as an autosomal dominant trait in the rat. *Toxicol. Lett.* 50:49-56.
- Pohjanvirta, R. (1991) Studies on the mechanism of acute toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in rats. Publication of National Public Health Institute, Helsinki, Finland. No. A1/1991.
- Pohjanvirta, R.; Tuomisto, J. (1987) Han/Wistar rats are exceptionally resistant to TCDD. *Arch. Toxicol.* 11:344-347.
- Pohjanvirta, R.; Juvonen, R.; Kärenlampi, S.; Raunio, H.; Tuomisto, J. (1988) Hepatic Ah-receptor levels and the effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on hepatic microsomal monooxygenase activity in a TCDD-susceptible and -resistant rat strain. *Toxicol. Appl. Pharmacol.* 92:131-140.
- Pohjanvirta, R.; Tuomisto, L.; Tuomisto, J. (1989) The central nervous system may be involved in TCDD toxicity. *Toxicology* 58:167-174.
- Pohjanvirta, R.; Håkansson, H.; Juvonen, R.; Tuomisto, J. (1990) Effects of TCDD on vitamin A status and liver microsomal enzyme activities in a TCDD-susceptible and a TCDD-resistant rat strain. *Food Chem. Toxic.* 28:197-203.
- Poiger, H.; Schlatter, C.H. (1980) Influence of solvents and adsorbents on dermal and intestinal absorption of TCDD. *Food. Cosmet. Toxicol.* 18:477-481.
- Poland, A.; Glover, E. (1973) Chlorinated dibenzo-*p*-dioxins: potent inducers of 6-aminolevulinic acid synthetase and aryl hydrocarbon hydroxylase. II. A study of the structure-activity relationship. *Mol. Pharmacol.* 9:736-747.

DRAFT--DO NOT QUOTE OR CITE

- Poland, A.; Glover, E. (1974) Comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a potent inducer of aryl hydrocarbon hydroxylase, with 3-methylcholanthrene. *Mol. Pharmacol.* 10:349-359.
- Poland, A.; Knutson, J.C. (1982) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanisms of toxicity. *Ann. Rev. Pharmacol. Toxicol.* 22:517-554.
- Poland, A.; Glover, E.; Kende, A.S. (1976) Stereospecific, high-affinity binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by hepatic cytosol. Evidence that the binding species is receptor for induction of aryl hydrocarbon hydroxylase. *J. Biol. Chem.* 251:4936-4946.
- Potter, C.L.; Menahan, L.A.; Peterson, R.E. (1986) Relationship of alterations in energy metabolism to hypophagia in rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Fundam. Appl. Toxicol.* 6:89-97.
- Puhvel, S.M.; Sakamoto, M.; Ertl, D.C.; Reisner, R.M. (1982) Hairless mice as models for chloracne: a study of cutaneous changes induced by topical application of established chloracnogens. *Toxicol. Appl. Pharmacol.* 64:492-503.
- Puhvel, S.M.; Connor, M.J.; Sakamoto, M. (1991) Vitamin A deficiency and the induction of cutaneous toxicity in murine skin by TCDD. *Toxicol. Appl. Pharmacol.* 107:106-116.
- Quilley, C.P.; Rifkind, A.B. (1986) Prostaglandin release by the chick embryo heart is increased by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and by other cytochrome P-448 inducers. *Biochem. Biophys. Res. Commun.* 136(2):582-589.
- Rifkind, A.B.; Gannon, M.; Gross, S.S. (1990) Arachidonic acid metabolism by dioxin-induced cytochrome P-450: a new hypothesis on the role of P-450 in dioxin toxicity. *Biochem. Biophys. Res. Commun.* 172(3):1180-1188.
- Roberts, L. (1991) Dioxin risks revisited. *Science* 251:624-626.
- Rozman, K.; Weber, L.W.D.; Pfeiffer, B.; et al. (1990). Evidence for an indirect mechanism of acute toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. *Organohalogen compounds, Vol. 1. Dioxin* 90.

DRAFT--DO NOT QUOTE OR CITE

- Safe, S. (1990) Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds; environmental and mechanistic considerations which support the development of toxicity equivalency factors (TEFs). *CRC Crit. Rev. Toxicol.* 21(1):51-88.
- Safe, S.; Astroff, B.; Harris, M.; Zacharewski, T.; Dickerson, R.; Romkes, M.; Biegel, L. (1991) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds as antioestrogens: characterization and mechanism of action. *Pharmacol. Toxicol.* 69:400-409.
- Schantz, S.L.; Barsotti, D.A.; Allen, J.R. (1978) Toxicological effects produced in nonhuman primates chronically exposed to fifty parts per trillion 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Toxicol. Appl. Pharmacol.* 48(1):A180.
- Schwetz, B.A.; Norris, J.M., Sparschu, G.L.; Rowe, V.K.; Gehring, P.J.; Emerson, J.L.; Gehring, C.G. (1973) Toxicology of chlorinated dibenzo-*p*-dioxins. *Environ. Health Perspect.* 5:87-99.
- Seefeld, M.D.; Peterson, R.E. (1983) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin-induced weight loss: a proposed mechanism. In: Human and environmental risks of chlorinated dioxins and related compounds. Tucker, R.E.; Young, A.L.; Gray, A.P., eds. *Environ. Sci. Res.* 26:405-412.
- Seefeld, M.D.; Peterson, R.E. (1984) Digestible energy and efficiency of feed utilization in rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* 74:214-222.
- Seefeld, M.D.; Corbett, S.W.; Keesey, R.E.; Peterson, R.E. (1984a) Characterization of the wasting syndrome in rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* 73:311-322.
- Seefeld, M.D.; Keesey, R.E.; Peterson, R.E. (1984b) Body weight regulation in rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* 76:526-536.
- Shen, E.S.; Gutman, S.I.; Olson, J.R. (1991) Comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated hepatotoxicity in C57BL/6J and DBA/2J mice. *J. Toxicol. Environ. Health* 32:367-381.
- Smith, A.G.; Francis, J.E.; Kay, S.J.E.; Greig, J.B. (1981) Hepatic toxicity and uroporphyrinogen decarboxylase activity following a single dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to mice. *Biochem. Pharmacol.* 30:2825-2830.

DRAFT--DO NOT QUOTE OR CITE

- Stahl, B.U.; Rozman, K. (1990) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)-induced appetite suppression in the SD rat is not a direct effect on feed intake regulation in the brain. *Toxicol. Appl. Pharmacol.* 106:158-162.
- Stohs, S.J.; Shara, M.A.; Alsharif, N.Z.; Wahba, Z.Z.; Al-Bayati, Z.A.F. (1990) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin-induced oxidative stress in female rats. *Toxicol. Appl. Pharmacol.* 106:126-135.
- Sunahara, G.I.; Lucier, G.W.; McCoy, Z.; Bresnick, E.H.; Sanchez, E.R.; Nelson, K.G. (1989) Characterization of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated decreases in dexamethasone binding to rat hepatic cytosolic glucocorticoid receptor. *Mol. Pharmacol.* 36:239-247.
- Thunberg, T. (1984) Effects of TCDD on vitamin A and its relation to TCDD toxicity. In: Banbury report 18. Poland, A.; Kimbrough, R.D., eds. Cold Spring Harbor Laboratory. Plainview, NY. pp. 333-344.
- Thunberg, T.; Håkansson, H. (1983) Vitamin A (retinol) status in the Gunn rat: the effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Arch. Toxicol.* 53:225-233.
- Thunberg, T.; Ahlberg, U.G.; Johnsson, H. (1979) Vitamin A (retinol) status in the rat after a single oral dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Arch. Toxicol.* 42:265-274.
- Toth, K.; Somfai-Relle, S.; Sugár, J.; Bence, J. (1979) Carcinogenicity testing of herbicide 2,4,5-trichlorophenoxyethanol containing dioxin and of pure dioxin in Swiss mice. *Nature* 278:548-549.
- Tuomisto, J.; Pohjanvirta, R. (1987) The Long-Evans rat: a prototype of an extremely TCDD-susceptible strain variant. *Pharmacol. Toxicol.* 60(suppl. I):72.
- Turner, J.N.; Collins, D.N. (1983) Liver morphology in guinea pigs administered either pyrolysis products of a polychlorinated biphenyl transformer fluid or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* 67:417-429.
- Umbreit, T.H.; Gallo, M.A. (1988) Physiological implications of estrogen receptor modulation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Lett.* 42:5-14.
- U.S. EPA. (1984) Ambient Water Quality Criteria for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Office of Water Regulations and Standards, Criteria and Standards Division, Washington, DC. EPA 440/5-84-007.

DRAFT--DO NOT QUOTE OR CITE

- U.S. EPA. (1985) Health Effects Assessment for polychlorinated dibenzo-*p*-dioxins. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH, for the Office of Emergency and Remedial Response, Washington, DC. EPA 600/8-84/0146.
- U.S. EPA. (1990) Drinking Water Criteria Document for polychlorinated biphenyls (PCBs). Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH, for the Office of Drinking Water, Washington, DC. ECAO-CIN 4-414.
- Van Miller, J.P.; Lalich, J.J.; Allen, J.R. (1977) Increased incidence of neoplasms in rats exposed to low levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Chemosphere* 61:625-632.
- Vos, J.G.; Beems, R.B. (1971) Dermal toxicity studies of technical polychlorinated biphenyls and fractions thereof in rabbits. *Toxicol. Appl. Pharmacol.* 19:617-633.
- Vos, J.G.; Koeman, J.H. (1970) Comparative toxicologic study with polychlorinated biphenyls in chickens with special reference to porphyria, edema formation, liver necrosis, and tissue residues. *Toxicol. Appl. Pharmacol.* 17:656-668.
- Vos, J.G.; Moore, J.A.; Zinkl, J.G. (1973) Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the immune system of laboratory animals. *Environ. Health Perspect.* 5:149-162.
- Vos, J.G.; Moore, J.A.; Zinkl, J.B. (1974) Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in C57B1/6 mice. *Toxicol. Appl. Pharmacol.* 29:229-241.
- Wahba, Z.Z.; Murray, W.J.; Hassan, M.Q.; Stohs, S.J. (1989a) Comparative effects of pair-feeding and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on various biochemical parameters in female rats. *Toxicology* 59:311-323.
- Wahba, Z.Z.; Lawson, T.W.; Murray, W.J.; Stohs, S.J. (1989b) Factors influencing the induction of DNA single strand breaks in rats by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Toxicology* 58:57-69.
- Wahba, Z.Z.; Murray, W.J.; Stohs, S.J. (1990a) Desferrioxamine-induced alterations in hepatic iron distribution, DNA damage, and lipid peroxidation in control and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated rats. *J. Appl. Toxicol.* 10(2):119-124.

- Wahba, Z.Z.; Murray, W.J.; Stohs, S.J. (1990b) Altered hepatic iron distribution and release in rats after exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Bull. Environ. Contam. Toxicol. 45:436-445.
- Walden, R.; Schiller, C.M. (1985) Short communications. Comparative toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in four (sub)strains of adult male rats. Toxicol. Appl. Pharmacol. 77:490-495.
- Wærn, F.; Flodström, S.; Busk, L.; Kronevi, T.; Nordgren, I.; Ahlborg, U.G. (1991a) Relative liver tumor promoting activity and toxicity of some polychlorinated dibenzo-*p*-dioxin and dibenzofuran congeners in female SD rats. Pharmacol. Toxicol. 69(6):450-458.
- Wærn, F.; Manzoor, E.; Ahlborg, U.G.; Håkansson, H. (1991b) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the lactating rat on maternal and neonatal vitamin A status and hepatic enzyme induction: a dose-response study. Chemosphere 23:1951-1956.
- Weber, L.W.D.; Lebofsky, M.; Stahl, B.U.; Gorski, J.R.; Muzi, G.; Rozman, K. (1991) Reduced activities of key enzymes of gluconeogenesis as possible cause of acute toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in rats. Toxicology 66:133-144.
- WHO/IPCS (World Health Organization/International Programme on Chemical Safety). (1989) Polychlorinated dibenzo-*p*-dioxins and dibenzofurans. Environmental Health Criteria 88.
- WHO/IPCS (World Health Organization/International Programme on Chemical Safety). (1991) Polychlorinated biphenyls (PCBs). Polychlorinated terphenyls (PCTs). Environmental Health Criteria. (in press)
- Wiebel, F.J.; Klose, U.; Kiefer, F. (1991) Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in vitro: H4IEC3-derived 5L hepatoma cells as a model system. Toxicol. Lett. 55:161-169.
- Yang, K.H.; Croft, W.A.; Peterson, R.E. (1977) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on plasma disappearance and biliary excretion of foreign compounds in rats. Toxicol. Appl. Pharmacol. 40:485-496.

DRAFT--DO NOT QUOTE OR CITE

- Yang, K.H.; Choi, E.J.; Choe, S.Y. (1983a) Cytotoxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on primary cultures of adult rat hepatocytes. Arch. Environ. Contam. Toxicol. 12:183-188.
- Yang, K.H.; Yoo, B.S.; Choe, S.Y. (1983b) Effects of halogenated dibenzo-*p*-dioxins on plasma disappearance and biliary excretion of ouabain in rats. Toxicol. Lett. 15:259-264.
- Zacharewski, T.; Safe, L.; Safe, S.; Chittim, B.; Devault, D.; Wiberg, K.; Bergqvist, P.-A.; Rappe, C. (1989) Comparative analysis of polychlorinated dibenzo-*p*-dioxin and dibenzofuran congeners in Great Lakes fish extracts by gas chromatography-mass spectrometry and in vitro enzyme induction activities. Environ. Sci. Technol. 23:730-735.
- Zinkl, J.G.; Vos, J.G.; Moore, J.A.; Gupta, B.N. (1973) Hematologic and clinical chemistry effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in laboratory animals. Environ. Health Perspect. 5:111-118.

Chapter 4. Immunotoxicity of TCDD and Related Halogenated Aromatic Hydrocarbons

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

NOTICE

THIS DOCUMENT IS A PRELIMINARY DRAFT. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

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

DRAFT--DO NOT QUOTE OR CITE

DISCLAIMER

This document is an internal draft for review purposes only and does not constitute Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

4. IMMUNOTOXICITY OF TCDD AND RELATED HALOGENATED AROMATIC HYDROCARBONS

4.1. INTRODUCTION

Concern over the potential toxic effects of chemicals on the immune system arises from the critical role that the immune system plays in maintaining health. It is well recognized that suppressed immunological function can result in increased incidence and severity of infectious diseases as well as some types of cancer. Conversely, the inappropriate enhancement of immune function or the generation of misdirected immune responses can precipitate or exacerbate the development of allergic and autoimmune diseases. Thus, suppression as well as enhancement of immune function are considered to represent potential immunotoxic effects of chemicals.

The immune system consists of a complex network of cells and soluble mediators that interact in a highly regulated manner to generate immune responses of appropriate magnitude and duration. Consequently, comprehensive evaluation of immunotoxicity must include specific assessments of multiple functional parameters on a kinetic basis. In addition, because an immune response develops in a time-dependent manner relative to antigen exposure, the immunotoxicity of a chemical can be profoundly influenced by the timing of chemical exposure relative to antigen challenge. Consideration of these levels of complexity involved in immunotoxicology assessment is critical for interpretation of the effects of chemical exposure on immune function.

Extensive evidence has accumulated over the past 20 years to demonstrate that the immune system is a target for toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and structurally **related** halogenated aromatic hydrocarbons (HAHs), including the polychlorinated dibenzofurans (**PCDFs**), polychlorinated biphenyls (PCBs), and polybrominated biphenyls (PBBs). This evidence was derived from numerous studies in various animal species, primarily rodents, but also guinea pigs, rabbits, monkeys, marmosets, and cattle. Epidemiological studies also provide evidence for the immunotoxicity of HAH in humans. In animals, relatively high doses of HAH produce lymphoid tissue depletion, except in the thymus where cellular depletion occurs at lower doses. Alterations in specific immune

effector functions and increased susceptibility to infectious disease have been identified at doses of TCDD well below those which cause lymphoid tissue depletion. Both cell-mediated and humoral immune responses are suppressed following TCDD exposure, suggesting that there are multiple cellular targets within the immune system that are altered by TCDD. Evidence also suggests that the immune system is indirectly targeted by TCDD-induced changes in nonlymphoid tissues. In addition, in parallel with increased understanding of the cellular and molecular mechanisms involved in immunity, studies on TCDD are beginning to establish biochemical and molecular mechanisms of TCDD immunotoxicity. These advances will be highlighted in this document.

There is an enormous literature based on descriptive studies on the immunotoxic effects of TCDD and related HAH in laboratory animals. Unfortunately, due to widely differing experimental designs, exposure protocols, and immunologic assays used, it has been very difficult to define a "TCDD-induced immunotoxic syndrome" in a single species, let alone across species. Until recently (Smialowicz et al., 1994), there was only one report that directly compared the effects of TCDD on the immune system of rats, mice, and guinea pigs. and even then, different immunologic parameters were assessed, and different antigens were used in the different species (Vos et al., 1973). In that study, the delayed-type hypersensitivity (DTH) response to tuberculin was evaluated in guinea pigs and rats for assessment of cell-mediated immunity, while the graft versus host (GVH) response was measured in mice. A decreased DTH response to tuberculin was observed in guinea pigs following 8 weekly doses of 40 ng/kg TCDD (total dose, 320 ng/kg), while the DTH response of rats to tuberculin was unaffected by 6 weekly doses of 5 µg/kg TCDD (total dose, 30,000 ng/kg). The GVH response in mice was suppressed by 4 weekly doses of 5 µg/kg TCDD (total dose, 20,000 ng/kg). The greater sensitivity of guinea pigs compared with rats and mice to the immunosuppressive effects of TCDD is consistent with the greater sensitivity of guinea pigs to other toxic effects of TCDD (McConnell et al., 1978; Poland and Knutson, 1982). Although these results appear to suggest that cell-mediated immunity in mice is more sensitive to TCDD than in rats, no studies have directly compared cell-mediated immunity in rats and mice using the same antigens and end points. There is, however, a recent study in which a direct comparison was made of the effects that TCDD

DRAFT--DO NOT QUOTE OR CITE

has on humoral immunity in rats and mice (Smialowicz et al., 1994). In this study the primary plaque-forming cell response to sheep red blood cells (SRBCs) was suppressed in B6C3F₁ mice (ED₅₀ of 0.68 µg/kg TCDD), while the anti-SRBC response was enhanced in Fischer 344 rats at a dose as high as 30 µg/kg TCDD. In another study in mice, the DTH response to oxazolone was suppressed by 4 weekly doses of 4 µg/kg TCDD (total dose, 16,000 ng/kg), while the DTH response to SRBC was unaffected by a tenfold higher dose of TCDD (Clark et al., 1981), illustrating that DTH responses to different antigens are not equally sensitive to TCDD-induced suppression, even in the same species. When PCB and PBB studies are considered, variable effects on DTH and other immune reactions are also apparent (Fraker, 1980; Vos and van Driel-Grootenhuis, 1972; Luster et al., 1980b; Thomas and Hinsdill, 1978). Because the exact basis for the interstudy variability is not known, it would serve no useful purpose in terms of risk assessment to catalog all of the effects of TCDD and other HAHs on the immune system that have been reported. Several comprehensive reviews have been published on the immunotoxic effects of HAH in general (Kerkvliet, 1984; Vos and Luster, 1989) and TCDD in particular (Holsapple et al., 1991a,b). The reader is also referred to the previous EPA TCDD risk assessment document, Appendix E (Sonawane et al., 1988) for another perspective on TCDD immunotoxicity. The present document will not reiterate this extensive literature, but rather, will emphasize more recent developments in the field of HAH immunotoxicity that may assist in the risk assessment process. Gaps in our knowledge that require further research will also be identified.

4.2. ROLE OF THE AH LOCUS IN HAH IMMUNOTOXICITY

One of the most important advances in the study of HAH toxicity in recent years has been the elucidation of a genetic basis for sensitivity to the toxicity of these chemicals, which may ultimately provide a logical explanation for much of the controversial data in the literature regarding HAH toxicity in different species and in different tissues of the same species. In this regard, many of the biochemical and toxic effects of HAH appear to be mediated via binding to an intracellular protein known as the aromatic hydrocarbon (Ah) or TCDD receptor in a process similar to steroid hormone receptor-mediated responses (Poland and Knutson, 1982; Cuthill et al., 1988). Ah receptor activation follows stereospecific

DRAFT--DO NOT QUOTE OR CITE

ligand binding; interaction of the receptor-ligand complex with dioxin-response elements (DREs) in the genome induces the transcription of the structural genes encoding mRNA for CYP1A1 enzyme activity (i.e., CYP1A1) as well as the expression of additional unidentified genes, the products of which are hypothesized to mediate HAH toxicity (Whitlock, 1990). Differences in toxic potency between various HAH congeners generally correlate with differences in Ah receptor-binding affinities. The most toxic HAH congeners are approximate stereoisomers of 2,3,7,8-TCDD and are halogen substituted in at least three of the four lateral positions in the aromatic ring system.

In mice, allelic variation at the Ah locus has been described (Poland et al., 1987; Poland and Glover, 1990). The different alleles code for Ah receptors that differ in their ability to bind TCDD and thus help to explain the different sensitivities of various inbred mouse strains to TCDD toxicity. Ah^{bb} C57Bl/6 (B6) mice represent the prototype "responsive" strain and are the most sensitive to TCDD toxicity, while Ah^{dd} DBA/2 (D2) mice represent the prototypic "nonresponsive" strain and require higher doses of TCDD to produce the same toxic effect. Recently, congenic Ah^{dd} mice on a B6 background were derived that differ from conventional B6 mice primarily at the Ah locus. The spectrum of biochemical and toxic responses to TCDD exposure was similar in both strains, but the doses needed to bring about the responses were significantly higher in congenic mice homozygous for the Ah^d allele as compared with mice carrying two Ah^b alleles (Birnbaum et al., 1990; Kerkvliet et al., 1990a).

Two lines of evidence have been used to investigate the Ah receptor dependence of the acute immunotoxicity of TCDD and related HAH: (1) comparative studies using PCDD, PCDF, and PCB congeners that differ in their binding affinity for the Ah receptor and (2) studies using mice of different genetic background known to differ at the Ah locus. Vecchi et al. (1983) were the first to report that the antibody response to sheep erythrocytes (SRBCs) was differentially suppressed by TCDD in B6 mice as compared with D2 mice, with D2 mice requiring approximately a 10 times higher dose to produce the same degree of suppression. Immunosuppression in F1 and backcross mice supported the role of the Ah locus in the expression of TCDD immunotoxicity. 2,3,7,8-TCDF was significantly less potent than TCDD and showed a similar differential immunosuppressive effect in B6 and D2

DRAFT--DO NOT QUOTE OR CITE

mice. At the **same** time, Silkworth and Grabstein (1982) reported a B6 versus D2 strain-dependent difference in sensitivity to suppression of the anti-SRBC response by 3,4,3',4'-tetrachlorobiphenyl, a ligand for the Ah receptor. In comparison, the 2,5,2',5'-tetrachlorobiphenyl isomer, which lacks affinity for the Ah receptor, was not immunosuppressive in either B6 or D2 mice. Structure-activity relationships were extended by Kerkvliet et al. (1985) in studies that compared the immunosuppressive potency of the chlorinated dioxin and furan isomers that contaminate technical grade pentachlorophenol. The 1,2,3,6,7,8-hexachlorinated dibenzo-*p*-dioxin (HxCDD), 1,2,3,4,6,7,8-heptachlorinated dibenzo-*p*-dioxin (HpCDD), and 1,2,3,4,6,7,8-heptachlorinated dibenzofuran (HpCDF) isomers, which bind the receptor, were all significantly immunosuppressive. The dose of each isomer that produced 50 percent suppression of the anti-SRBC response (ID₅₀) was 7.1, 85, and 208 µg/kg for HxCDD, HpCDD, and HpCDF, respectively (Figure 4-1). The ID₅₀ for TCDD was 0.65 µg/kg based on the data of Vecchi et al. (1980). In contrast, octachlorodibenzo-*p*-dioxin (OCDD), which does not bind the receptor, was not immunosuppressive at a dose as high as 500 µg/kg (Kerkvliet et al., 1985). More extensive structure-dependent immunosuppressive activities of technical grade PCB mixtures (Davis and Safe, 1990), PCB congeners (Davis and Safe, 1989), and PCDF congeners (Davis and Safe, 1988) have also been reported. Results of these studies using different HAH congeners are summarized in Table 4-1.

The role of the Ah receptor in suppression of the anti-SRBC response has recently been verified in studies using B6 mice congenic at the Ah locus (Kerkvliet et al., 1990a). As expected, congenic Ah^{dd}-B6 mice were significantly less sensitive to TCDD-induced immune suppression as compared with wild-type Ah^{bb}-B6 mice. Unexpectedly, however, the dose response in **congenic** B6-Ah^{dd} mice appeared to be bimodal, with a portion of the response sensitive to **suppression** by low doses of TCDD. Because of the bimodal response, the data did not permit extrapolation of an ID₅₀ dose in the congenic mice. The results were interpreted to suggest potential non-Ah receptor-mediated immunosuppressive effects. It should be noted, however, that recent studies by Silkworth et al. (1993) using rederived congenic Ah^{dd}-B6 mice have not corroborated a bimodal dose response. The issue of Ah

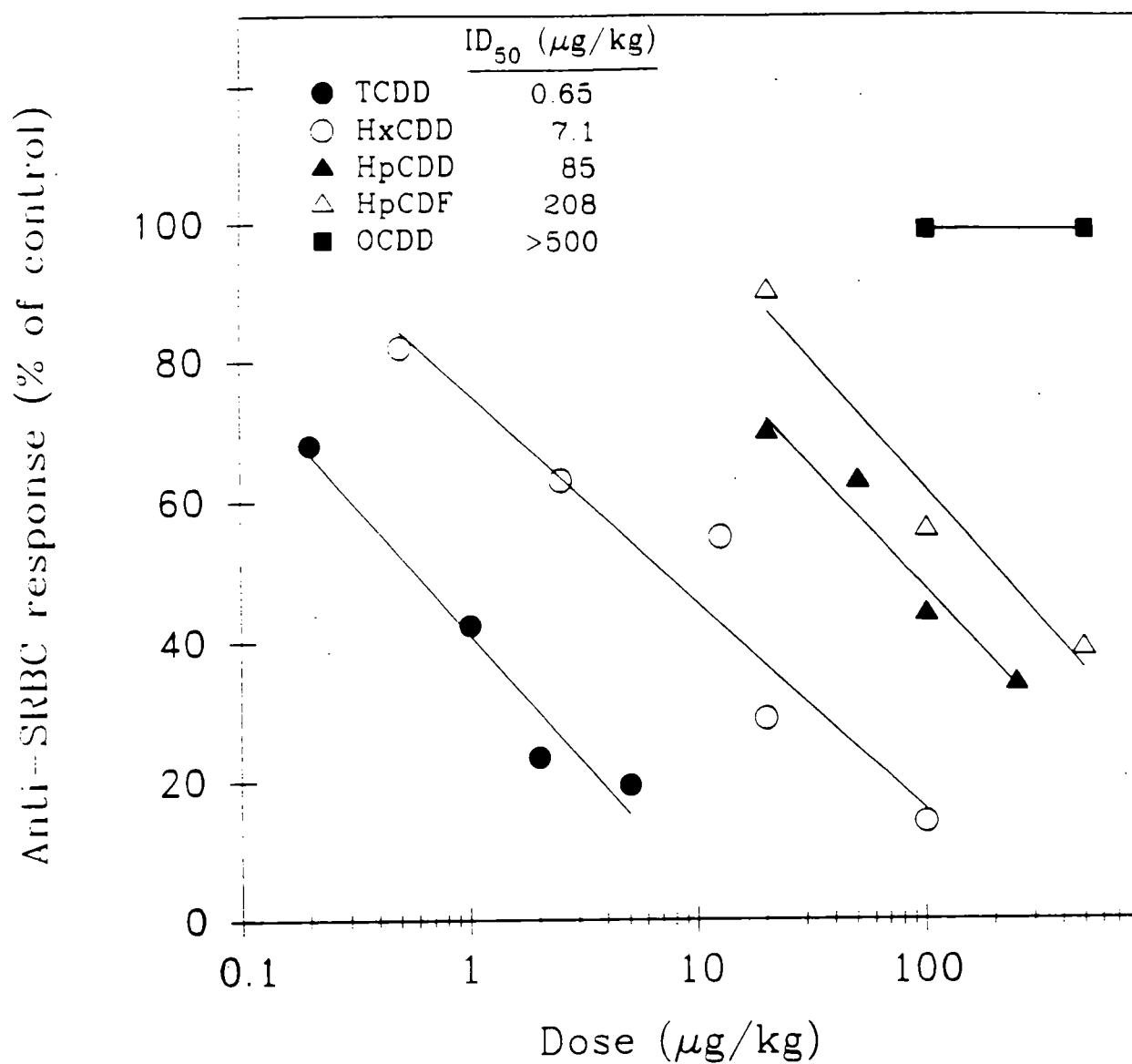


Figure 4-1. Structure-dependent immunotoxicity of some polychlorinated dioxin and furan isomers. Immunotoxicity assessed by suppression of the splenic antibody response to SRBC (modified from Kerkvliet et al., 1985).

DRAFT--DO NOT QUOTE OR CITE

Table 4-1. Toxic Equivalency Factors (TEFs) for Polychlorinated Dioxins, Furans, and Biphenyls Based on the Acute Single Dose ID₅₀ for Suppression of the PFC Response to SRBC in Ah-responsive B6 Mice

Congener	ID ₅₀	Reference	TEF
2,3,7,8-TCDD	0.74 µg/kg	Kerkvliet and Brauner, 1990a	1.0
"	0.65 µg/kg	Vecchi et al., 1980	(based on mean ID ₅₀ of 0.7 ± 0.07 µg/kg)
"	0.77 µg/kg	Davis and Safe, 1988	
"	0.60 µg/kg	Kerkvliet et al., 1990a	
1,2,3,6,7,8-HxCDD	7.1 µg/kg	Kerkvliet et al., 1985	0.1
1,2,3,4,6,7,8-HpCDD	85.0 µg/kg	Kerkvliet et al., 1985	8.2 × 10 ⁻³
OCDD	>500 µg/kg	Kerkvliet et al., 1985	<1.4 × 10 ⁻³
2,3,4,7,8-PCDF	1.0 µg/kg	Davis and Safe, 1988	0.7
2,3,7,8-TCDF	4.3 µg/kg	Davis and Safe, 1988	1.6 × 10 ⁻¹
1,2,3,4,6,7,8-HpCDF	208 µg/kg	Kerkvliet et al., 1985	3.4 × 10 ⁻³
1,2,3,7,9-PeCDF	239 µg/kg	Davis and Safe, 1988	2.9 × 10 ⁻³
1,3,6,8-TCDF	11 mg/kg	Davis and Safe, 1988	6.4 × 10 ⁻⁵
3,4,3',4'-TCB	28 mg/kg ^a	Silkworth and Grabstein, 1982	2.5 × 10 ⁻⁵
2,3,4,5,3',4'-HxCB	0.7 mg/kg	Davis and Safe, 1990	1.0 × 10 ⁻³
2,3,4,5,3',4'-HxCB	36 mg/kg ^a	Silkworth et al., 1984	1.9 × 10 ⁻⁵
2,4,3',4',5',6'-HxCB	43 mg/kg	Davis and Safe, 1990	1.6 × 10 ⁻⁵
2,3,4,3',5'-PeCB	65 mg/kg	Ibid.	1.1 × 10 ⁻⁵
2,3,4,5,3',5'-HxCB	72 mg/kg	Ibid.	9.7 × 10 ⁻⁶
2,4,2',4'-TCB	>100 mg/kg	Silkworth et al., 1984	<7.0 × 10 ⁻⁶
2,4,5,2',4',6'-HxCB	>360 mg/kg	Davis and Safe, 1990	<1.9 × 10 ⁻⁶
2,4,6,2',4',6'-HxCB	>360 mg/kg	Ibid.	<1.9 × 10 ⁻⁶
2,4,5,2',4',5'-HxCB	>360 mg/kg	Biegel et al., 1989	<1.9 × 10 ⁻⁶
Aroclor 1260	104 mg/kg	Davis and Safe, 1989	6.7 × 10 ⁻⁶

DRAFT--DO NOT QUOTE OR CITE

Table 4-1. (continued)

Congener	ID ₅₀	Reference	TEF
Aroclor 1254	118 mg/kg	Ibid.	5.9×10^{-6}
Aroclor 1254	207 mg/kg	Lubet et al., 1986	3.4×10^{-6}
Aroclor 1248	190 mg/kg	Davis and Safe, 1989	3.7×10^{-6}
Aroclor 1242	391 mg/kg	Ibid.	1.8×10^{-6}
Aroclor 1016	408 mg/kg	Ibid.	1.7×10^{-6}
Aroclor 1232	464 mg/kg	Ibid.	1.5×10^{-6}

^a Interpolated from two data points.

receptor-independent immunotoxicity will be discussed in detail in a subsequent section of this document.

Ah receptor dependency of HAH immunotoxicity has also been demonstrated in mice using other immunologic responses. For example, Kerkvliet et al. (1990a) reported that the ID₅₀ for suppression of the antibody response to TNP-LPS in Ah^{bb}-B6 mice was 7.0 µg/kg compared with a significantly higher ID₅₀ of 30 µg/kg in congenic Ah^{dd}-B6 mice. Because the antibody response to TNP-LPS shows little requirement for macrophages or T helper cells (Jelinek and Lipsky, 1987), these results suggest an Ah receptor-dependent B cell response. In terms of cytotoxic T cells, Clark et al. (1983) was first to report data suggesting that TCDD and PCB isomers suppressed in vitro cytotoxic T lymphocyte (CTL) responses of B6 and D2 mice through an Ah receptor-dependent mechanism. Subsequently, Kerkvliet et al. (1990b) reported that B6 mice congenic at the Ah locus showed Ah-dependent sensitivity to suppression of the CTL response following exposure to either TCDD or 3,4,5,3',4',5'-hexachlorobiphenyl (HxCB). Furthermore, the potency of TCDD and of three HxCB congeners to suppress the CTL response of Ah^{bb}-B6 mice directly correlated with their relative binding affinities for the Ah receptor (Table 4-2, from Kerkvliet et al., 1990b). The ID₅₀ of TCDD for suppression of the CTL response in B6 mice was 7.0 µg/kg.¹

In summary, the data relating HAH immunotoxicity, at least in part, to Ah receptor-dependent events are convincing. However, it should be emphasized that all of the data have been obtained from studies in inbred mice using an acute or subacute exposure regimen. Except for thymic atrophy, structure-immunotoxicity relationships in other species, including rats, have not been established, and the availability of inbred strains of other species with defined Ah genotype are not currently available. The importance of Ah

¹The dose of TCDD required to suppress the CTL response reported by Kerkvliet et al. (1990b) is significantly greater than that reported by Clark et al. (1981), who reported CTL suppression following 4 weekly doses of 0.1 µg/kg TCDD. Clark et al. (1983) also reported that doses of TCDD as low as 4 ng/kg to B6 mice suppressed the in vitro generation of CTL and that the suppression was Ah dependent. The potency of TCDD described in Clark's studies has not been corroborated by other laboratories. For example, the in vivo and in vitro CTL response in B6 mice was not affected at doses ranging from 0.01 to 3.0 µg/kg at weekly intervals for 4 weeks (Hanson and Smialowicz, 1994).

DRAFT--DO NOT QUOTE OR CITE

Table 4-2. TEFs Based on the ID₅₀ for Suppression of Alloantigen (P815)-Specific CTL Response in C57Bl/6 Mice

Congener	ID ₅₀	Reference	TEF
TCDD	7 µg/kg	Kerkvliet et al., 1990b	1.0
3,4,5,3',4',5'-HxCB	7 mg/kg	Ibid.	1×10^{-3}
2,3,4,5,3',4'-HxCB	70 mg/kg ^a	Ibid.	1×10^{-4}
2,4,5,2',4',5'-HxCB	> 300 mg/kg	Ibid.	$< 2.3 \times 10^{-5}$

^a Interpolated from two data points.

receptor-mediated events in chronic, low-level HAH immunotoxicity also remains to be established. Morris et al. (1992) have recently reported that the sensitivity of D2 mice to TCDD-induced suppression of the anti-SRBC response increased significantly when TCDD was administered daily over 2 weeks rather than as an acute single dose. Unfortunately, in these studies, the lowest dose of TCDD produced near-maximum suppression of the anti-SRBC response of B6C3F₁ mice in the acute exposure model, precluding the detection of any similar increase in sensitivity of the B6C3F₁ mice to chronic dosing. In contrast to these findings, Vecchi et al. (1983) reported that multiple exposures to TCDD (2 µg/kg for 5 weeks or 0.5 µg/kg for 8 weeks) did not increase the sensitivity of D2 mice to suppression of the anti-SRBC response. Thus, the basis for any change in potency resulting from multiple treatment or chronic exposure to TCDD and the role of Ah receptor-mediated events in the phenomenon remain to be elucidated.

4.3. TOXIC EQUIVALENCY FACTORS (TEF) FOR IMMUNOTOXICITY

Based on the available data from mice, the majority of the immunotoxic effects of HAH appear to be mediated via the Ah receptor. Thus, the toxicity of different HAH congeners can be compared by calculating TCDD-equivalence factors (TEF). TEFs based on acute, single-dose exposure (oral or i.p.) of B6 mice to various HAH for suppression of the anti-SRBC response and the CTL response are presented in Tables 4-1 and 4-2, respectively. As shown in Table 4-1, the potency of TCDD to suppress the antibody response to SRBC has been reported by several laboratories, with remarkable agreement² in the ID₅₀ value of 0.7 µg/kg in B6 mice. The ID₅₀ of B6C3F₁ mice is similar (< 1 µg/kg; House et al., 1990:

²Several laboratories have reported that the antibody response to SRBC is sensitive to suppression following acute exposure to TCDD, either i.p. or orally, at doses <1 µg/kg. In contrast, Clark et al. (1981) reported that 4 weekly i.p. doses of 10 but not 1 or 0.1 µg/kg TCDD significantly suppressed the anti-SRBC response in B6 mice. The chronic-dosing protocol used by Clark does not readily explain his decreased potency because Vecchi et al. (1983) reported that 5 weekly doses of 2 µg/kg or 8 weekly doses of 0.5 µg/kg TCDD significantly suppressed the anti-SRBC response. Likewise, when total doses of 0.2 or 1 µg/kg TCDD were given as a single dose or divided into 5 daily doses, the divided dose produced slightly more suppression than the single dose (see Table 4-3). In addition, the route of antigen challenge (intravenous [used by Clark] vs. intraperitoneal [used by Vecchi]) does not appear to greatly influence the degree of suppression of the anti-SRBC response produced by TCDD (see Table 4-4). Thus, the basis for the discrepancies between the data of Clark et al. (1981, 1983) and other laboratories regarding the potency of TCDD to suppress the anti-SRBC response is unknown.

DRAFT--DO NOT QUOTE OR CITE

Table 4-3. Effect of Single vs. Multiple Dosing With TCDD On Suppression of the Antibody Response to SRBC in C57Bl/6 Mice

Total Dose ($\mu\text{g/kg}$)	Plaque-Forming Cells/ 10^6 Spleen Cells (Mean $\pm$ SD)	
	Single ^a	Multiple ^b
0	2,460 $\pm$ 657	3,846 $\pm$ 1618
0.2	1,879 $\pm$ 445 (76)	2,356 $\pm$ 592 (61)
1.0	1,293 $\pm$ 285 (52) ^c	1,143 $\pm$ 208 (30) ^c

^a Total dose of TCDD given once 2 days prior to SRBC injection.

^b Total dose of TCDD divided into five equal doses administered on days -7 to -2 prior to SRBC injection.

^c $p < 0.01$.

Table 4-4. Influence of Route of Antigen Challenge on Suppression of the Antibody Response to SRBC in C57Bl/6 Mice^a

Dose of TCDD ($\mu\text{g/kg}$)	Plaque-Forming Cells/ 10^6 Spleen Cells (Mean $\pm$ SD)	
	IV	IP
0	1,151 $\pm$ 367	1,812 $\pm$ 872
0.2	623 $\pm$ 324 (54)	1,197 $\pm$ 519 (66)
1.0	466 $\pm$ 212 (40) ^b	697 $\pm$ 163 (38) ^b

^a 2.5×10^8 SRBC were injected intravenously (IV) or intraperitoneally (IP) 2 days after oral dosing with TCDD. Plaque-forming cells were measured 5 days later.

^b $p < 0.01$.

Smialowicz et al., 1994) or slightly higher (1.2 $\mu\text{g/kg}$; Holsapple et al., 1986a) in comparison with B6 mice. These data thus provide a well-defined base value to use in calculating TEFs for other HAH congeners in the context of suppression of the anti-SRBC response.

However, in contrast to the reproducible data on TCDD, the accuracy of the derived TEFs for other HAH congeners shown in Table 4-1 is difficult to evaluate because few congeners have been examined in more than one study. In the few cases where the same congener has been evaluated independently, discrepancies in the data exist. For example, both Davis and Safe (1990) and Silkworth et al. (1984) evaluated the potency of the 2,3,4,5,3',4'-HxCB congener in the anti-SRBC response. Based on the ID_{50}s from the two data sets, the respective TEFs differ by almost two orders of magnitude (1×10^{-3} vs. 2×10^{-5}). When the same congener was compared with TCDD for suppression of the CTL response, the TEF was 1×10^{-4} (Table 4-2). The basis for these discrepancies is unknown. Thus, the database for TEF comparisons using immunotoxicity data must be expanded considerably before TEFs can be used with confidence in risk assessment.

4.4. INTERACTIONS BETWEEN HAH

If the immunotoxicity of TCDD and structurally related HAH is dependent on Ah receptor-mediated mechanisms, then coexposure to subsaturating levels of more than one Ah receptor ligand should produce additive effects. An additive interaction has been demonstrated in mice coexposed to 1,2,3,6,7,8-HxCDD and 1,2,3,4,6,7,8-HpCDD, two relatively strong Ah receptor ligands (Kerkvliet et al., 1985). On the other hand, Davis and Safe (1988, 1989) have reported that coexposure of mice to an immunotoxic dose of TCDD and a subimmunotoxic dose of different commercial Aroclors or different PCB congeners resulted in **partial antagonism** of TCDD suppression of the anti-SRBC response. In limited studies, an apparently similar antagonism was observed following coexposure to 2,3,7,8-TCDF (10 $\mu\text{g/kg}$) and TCDD (1.2 $\mu\text{g/kg}$) (Rizzardini et al., 1983). The mechanism for the antagonism has not been fully elucidated, but the effects are consistent with competition for binding at the Ah receptor because the weaker agonist was administered in excess compared with TCDD. Furthermore, Silkworth et al. (1988) and Silkworth and

O'Keefe (1992) have shown that the immunotoxicity of TCDD can be modified by coexposure to other HAH present as cocontaminants of actual environmental samples from Love Canal, New York. Such interactions complicate hazard assessment of mixtures based on TEFs and may preclude dependence on TEFs without biological response evaluation for risk assessment.

4.5. SENSITIVE TARGETS FOR HAH IMMUNOTOXICITY

Despite considerable investigation, the cells that are altered by HAH exposure leading to suppressed immune function have not been unequivocally identified. The main reason for the lack of definitive progress in this area is the conflicting data reported from different laboratories regarding the ability of TCDD to suppress lymphocyte functions when examined "ex vivo" or in vitro. As discussed in a subsequent section of this document, the in vitro effects of TCDD are greatly influenced by the in vitro culture conditions, which may explain the discrepancies in effects observed in different laboratories.

In contrast to in vitro studies, the in vivo immunotoxicity of TCDD, expressed in terms of suppression of the anti-SRBC response of B6 or B6C3F₁ mice, is highly reproducible between laboratories. Because the magnitude of the anti-SRBC response depends on the concerted interactions of antigen-presenting cells (APC), regulatory T cells (helper and suppressor), and B cells, this response has been used most widely to evaluate target cell sensitivity to HAH. In addition, the antibody response to SRBC can be modulated by many nonimmunological factors, including hormonal and nutritional variables, and HAH are known to affect numerous endocrine and metabolic functions. These latter effects will be apparent only in in vivo studies, while only direct effects of HAH on APC and lymphocyte functions would be evident following in vitro exposure to HAH. To date, direct in vitro effects of TCDD on purified B cell activity have been reported (Holsapple et al., 1986a; Morris et al., 1991; Luster et al., 1988), while direct effects on macrophages and T cells in vitro have not been described. (The in vitro effects of TCDD will be discussed in more detail in a subsequent section of this document.)

Kerkvliet and Brauner (1987) compared the sensitivity of antibody responses to antigens that differ in their requirements for APC and T cells as an in vivo approach to

DRAFT--DO NOT QUOTE OR CITE

evaluate the **cellular** targets of 1,2,3,4,6,7,8-HpCDD humoral immunotoxicity. The T helper cell independent (TI) antigens, DNP-Ficoll and TNP-LPS, were used in these studies. These TI antigens differ from each other in their requirement for APC (higher for DNP-Ficoll) and their sensitivity to regulatory (amplifier and suppressor) T cell influence (DNP-Ficoll is sensitive, TNP-LPS is not) (Braley-Mullen, 1982). Obviously, all antibody responses require B cell differentiation into antibody-secreting plasma cells. Although HpCDD produced dose-dependent suppression of the antibody response to all three antigens, sensitivity to suppression directly correlated with the sensitivity of the response to T cell regulation. The ID₅₀s were 53, 127, and 516 µg/kg for SRBC, DNP-Ficoll, and TNP-LPS, respectively. These results were interpreted as follows: If one assumes that B cell function is targeted in the TNP-LPS response, then regulatory T cells and/or APC may represent the more sensitive target in the SRBC and DNP-Ficoll responses. The difference in sensitivity between the SRBC and DNP-Ficoll responses suggests that the T helper cell may be a particularly sensitive target. The differential sensitivity of the antibody responses to TNP-LPS versus SRBC has been corroborated in TCDD-treated mice (House et al., 1990; Kerkvliet et al., 1990a). Thus, the exquisite in vivo sensitivity of the antibody response to SRBC would appear to depend on the T cell and/or APC components of the response rather than the B cell, unless the B cells that respond to SRBC are different from the B cells that respond to TNP-LPS. Currently, evidence for such a difference is lacking. However, this interpretation conflicts with the ex vivo data of Dooley and Holsapple (1988). Using separated spleen T cells, B cells, and adherent cells from vehicle- and TCDD-treated mice, they reported that B cells from TCDD-treated mice were functionally compromised in in vitro antibody responses but T cells and macrophages were not. The basis for these discrepant findings has not been established. However, it is possible that the effects of TCDD on T cells are indirectly induced following antigen exposure such that removal of the cells from the TCDD environment of the host prior to antigen challenge would preclude detection of T cell dysfunction. This interpretation is supported by the findings of Tomar and Kerkvliet (1991) that spleen cells taken from TCDD-treated mice were not compromised in their ability to reconstitute the antibody response of lethally irradiated mice. This

interpretation is **also** consistent with the reported lack of direct effects of TCDD and other HAH on T cells in vitro (Clark et al., 1981; Kerkvliet and Baecher-Steppan, 1988a).

Although the direct effects of TCDD on T cells in vitro have not been demonstrated, it is clear that functional T cell responses generated in vivo are compromised following in vivo exposure. Nude mice that are congenitally T cell deficient are significantly less sensitive to HpCDD-induced immunotoxicity when compared with their T cell-competent littermates (Kerkvliet and Brauner, 1987). Likewise, exposure to TCDD or HxCB suppresses the development of CTL activity following alloantigen challenge (Kerkvliet et al., 1990b). The influence of TCDD exposure on regulatory T cell functions has been addressed in a limited number of studies. Clark et al. (1981) first proposed that T suppressor cells were induced by TCDD in the thymus that were responsible for the suppressed CTL response. However, increased suppressor cell activity in peripheral lymphoid tissue was not observed in mice exposed to TCDD (Dooley et al., 1990) or 3,4,5,3',4',5'-HxCB (Kerkvliet and Baecher-Steppan, 1988b). In terms of T helper cell activity, Tomar and Kerkvliet (1991) reported that a dose of 5 $\mu\text{g/kg}$ TCDD suppressed the in vivo generation of carrier-specific T helper cells. Lundberg et al. (1990) reported that thymocytes from B6 mice treated with TCDD (50 $\mu\text{g/kg}$) were less capable of providing help for an in vitro anti-SRBC response. However, Clark et al. (1983) reported in ex vivo studies that T cells from TCDD-treated mice produced normal levels of IL-2. The in vivo effect of TCDD on the production of IL-2 as well as other lymphokines important in the development of an antibody response (e.g., IL-4, IL-5) have not been reported.

The influence of TCDD exposure on B cell function has been addressed primarily in in vitro studies. The issue is difficult to address in vivo given that most B cell responses (except perhaps anti-LPS responses) are dependent on interactions with T cells and macrophages. In vitro studies have described the direct effects of TCDD on the activation and differentiation of purified B cells (Luster et al., 1988; Morris et al., 1991). These studies suggest that TCDD inhibits the terminal differentiation of B cells via alteration of an early activation event (Luster et al., 1988). Increased phosphorylation and tyrosine kinase activity in TCDD-treated B cells may underlie this B cell dysfunction (Kramer et al., 1987; Clark et al., 1991a).

DRAFT--DO NOT QUOTE OR CITE

Macrophage functions have also been examined following TCDD exposure and generally found to be resistant to suppression by TCDD when assessed *ex vivo*. Macrophage-mediated phagocytosis, macrophage-mediated tumor cell cytolysis or cytostasis, oxidative reactions of neutrophils and macrophages, and natural killer (NK) cell activity were not suppressed following TCDD exposure, with doses as high as 30 $\mu\text{g/kg}$ failing to suppress NK and macrophage functions (Vos et al., 1978; Mantovani et al., 1980). A potentially important exception is the reported selective inhibition of phorbol ester-activated antitumor cytolytic and cytostatic activity of neutrophils by TCDD (Ackermann et al., 1989).

On the other hand, it is interesting to note that the pathology associated with TCDD toxicity often includes neutrophilia and an inflammatory response in liver and skin characterized by activated macrophage and neutrophil accumulation (Weissberg and Zinkl, 1973; Vos et al., 1973; Vos et al., 1974; Puhvel et al., 1988). Although these observations may simply reflect a normal inflammatory response to tissue injury, there is some preliminary experimental evidence that suggests inflammatory cells may be activated by TCDD exposure. For example, Alsharif et al. (1990) recently reported that TCDD increased superoxide anion production in rat peritoneal macrophages. In addition, it has been shown that TCDD exposure results in an enhanced inflammatory response following SRBC challenge (Kerkvliet and Brauner, 1990b; Kerkvliet and Oughton, 1993). This effect of TCDD was characterized by a twofold to fourfold increase in the number of neutrophils and macrophages locally infiltrating the intraperitoneal site of SRBC injection. However, the kinetics of the cellular influx was not altered by TCDD. Likewise, the expression of macrophage activation markers (I-A and F4/80) and the antigen-presenting function of the peritoneal exudate cells were unaltered by TCDD. Thus, the effect of TCDD appeared to reflect a quantitative rather than a qualitative change in the inflammatory response. Importantly, TCDD-induced suppression of the anti-SRBC response could not be overcome by increasing the amount of antigen used for sensitization, suggesting that enhanced antigen clearance/degradation by the increased numbers of phagocytic cells (e.g., decreased antigen load) was not responsible for the decreased antibody response in TCDD-treated mice. Thus, the relationship, if any, between the inflammatory and immune effects of TCDD remains to be elucidated.

DRAFT--DO NOT QUOTE OR CITE

One mechanism by which TCDD and related HAH may augment inflammatory responses is via enhanced production of inflammatory mediators such as interleukin 1 (IL-1) and tumor necrosis factor (TNF). Recent evidence suggests that the long-recognized hypersusceptibility of TCDD- and PCB-treated animals to endotoxin (lipopolysaccharide [LPS]) (Thomas and Hinsdill, 1978; 1979; Vos et al., 1978; Loose et al., 1979) may be related to an increased production of TNF and/or IL-6 in the chemically treated animals (Clark et al., 1991b; Taylor et al., 1990; Hoglen et al., 1992). The ability of methylprednisolone to reverse the mortality associated with TCDD/endotoxin treatment is also consistent with an inflammatory response (Rosenthal et al., 1989). Similarly, increased inflammatory mediator production may underlie the enhanced rat paw edema response to carrageenan and dextran in TCDD-treated rats (Theobald et al., 1983; Katz et al., 1984). Limited preliminary data are available to indicate that the production of inflammatory mediators such as TNF (Taylor et al., 1990; Clark et al., 1990b) and IL-6 (Hoglen et al., 1992) may be increased in HAH-treated animals. Serum complement activity, on the other hand, has been reported to be suppressed in dioxin-treated mice (White et al., 1986), although enhanced activity was reported at the lowest exposure level when 1,2,3,6,7,8-HxCDD was tested. A primary effect of TCDD on IL-1 is supported by the recent findings of Sutter et al. (1991) that the IL-1 β gene contains a DRE. Likewise, Stepan and Kerkvliet (1991) have reported that under some exposure conditions TCDD increased the level of mRNA for IL-1 in TCDD-treated IC21 cells, a macrophage cell line derived from B6 mice. On the other hand, House et al. (1990) reported that inflammatory macrophages obtained from TCDD-treated mice produced control levels of IL-1 when examined ex vivo. Thus, the effect of TCDD on inflammatory mediator production may be a "priming effect" and require coexposure to antigen or LPS. The influence of TCDD on inflammatory mediator production and action is an important area for further study.

Because the rapid influx of phagocytic cells to the site of pathogen invasion is an important factor in host resistance to infection, the ability of TCDD to augment the production of inflammatory chemoattractive mediators would imply that TCDD exposure could result in enhanced host resistance. However, since TCDD exposure is, at the same time, immunosuppressive, resulting in decreased specific immune responses generated by T

and B lymphocytes, the overall impact of TCDD exposure on disease susceptibility will likely vary depending on the nature of the pathogen and the major mode of host response to the specific infectious agent. Such effects may in fact help to explain the disparate effects of TCDD in different host resistance models that have been previously reported.

4.6. INFLUENCE OF TCDD ON HOST RESISTANCE TO DISEASE

The ability of an animal to resist and/or control viral, bacterial, parasitic, and neoplastic diseases is determined by both nonspecific and specific immunological functions. Decreased functional activity in any immunological compartment may result in increased susceptibility to infectious and neoplastic diseases. In terms of risk assessment, host resistance is often accorded the "bottom line" in terms of relevant immunotoxic end points. Animal host resistance models that mimic human disease are available and have been used to assess the effect of TCDD on altered host resistance.

TCDD exposure increases susceptibility to challenge with the gram-negative bacterium *Salmonella*. TCDD was given per os at 0.5 to 20 $\mu\text{g/kg}$ once a week for 4 weeks to male 4-week-old C57Bl/6Jfh (J67) mice and challenged 2 days after the fourth dose (when mice were 8 weeks old) with either *Salmonella bern* or *Herpesvirus suis* (also known as pseudorabies virus). Results with *S. bern* indicated that there was an increased mortality at 1 $\mu\text{g TCDD/kg}$ (total dose of 4 $\mu\text{g/kg}$) and a reduced time to death after bacterial challenge with 5 $\mu\text{g TCDD/kg}$ (total dose of 20 $\mu\text{g/kg}$). In contrast, the same doses of TCDD did not alter the time to death or the incidence of mortality following *Herpesvirus suis* infection (Thigpen et al., 1975). A TCDD feeding study by Hinsdill et al. (1980) also demonstrated increased susceptibility of 7-week-old Swiss Webster outbred female mice to *S. typhimurium* var. *copenhagen*. Mice were fed control feed or feed containing 10, 50, or 100 ppb TCDD for 8 weeks, after which they were injected intravenously with $10^{3.5}$ *S. typhimurium* var. *copenhagen*. Results indicated that 50 and 100 ppb TCDD increased mortality from *Salmonella* and shortened the time to death, while 10 ppb caused an increased bacteremia. Vos et al. (1978) reported that TCDD resulted in a reduced resistance to endotoxin (*E. coli* O 127:B 8 lipopolysaccharide) and suggested that the increased susceptibility to *Salmonella*

caused by TCDD may be due to the lipopolysaccharide or endotoxin of this gram-negative bacterium. Vos et al. (1978) demonstrated reduced resistance to endotoxin with a single oral dose of 100 μg TCDD/kg using 3- to 4-week-old outbred female mice and challenged with endotoxin 5 days later. Vos et al. (1978) also reported enhanced mortality from the intravenous injection of endotoxin 2 days after the final oral dose of TCDD (1.5, 5, 15, or 50 $\mu\text{g}/\text{kg}$; once a week for 4 weeks) in 3- to 4-week-old male outbred Swiss mice. These studies indicate a reduced resistance to endotoxin after single or multiple doses of TCDD. Thomas and Hinsdill (1979), using *S. typhimurium* lipopolysaccharide, demonstrated a reduced resistance to endotoxin in the offspring of female Swiss Webster mice fed TCDD prior to mating, during gestation, and between parturition and weaning. Rosenthal et al. (1989) used female B6C3F₁, DBA/2, as well as congenic mice to demonstrate that acute doses of 50, 100, or 200 μg TCDD per os increased endotoxin-induced mortality in B6C3F₁ mice, which was associated with hepatotoxicity and decreased clearance of the endotoxin. D2 and Ah^{dd} congenic mice were relatively resistant to this effect, implicating Ah receptor-dependent mechanisms in endotoxin hypersensitivity.

White et al. (1986) reported that *Streptococcus pneumoniae*, a gram-positive bacterium that does not contain endotoxin, caused increased mortality in 5- to 6-week-old female B6C3F₁ mice after subchronic oral administration of TCDD (1 $\mu\text{g}/\text{kg}$ for 14 days) and challenged with *S. pneumoniae* intraperitoneally 1 day after the last treatment. The 1,2,3,6,7,8 hexachlorodibenzo-*p*-dioxin (HCDD) isomer also resulted in a dose-dependent increase in susceptibility to *S. pneumoniae*.

Enhanced susceptibility to viral disease has also been reported after TCDD administration. Clark et al. (1983) injected TCDD intraperitoneally once a week for 4 weeks and challenged mice 7 to 22 days later with *Herpes simplex* type II strain 33 virus. Mice receiving TCDD at 0.04, 0.4, or 4.0 $\mu\text{g}/\text{kg}$ weekly (total dose of 160, 1,600, and 16,000 ng/kg) all had significantly enhanced mortality to Herpesvirus type II infection. House et al. (1990) also reported an enhanced susceptibility to viral infection following low-level single-dose TCDD administration intraperitoneally. B6C3F₁ female mice, 6 to 8 weeks of age, were challenged with influenza A/Taiwan/1/64 (H2N2) virus 7 to 10 days following TCDD

DRAFT--DO NOT QUOTE OR CITE

TCDD administration at 10, 1.0, and 0.1 $\mu\text{g/kg}$ decreased resistance to virus. The lowest observable effect level was 0.1 $\mu\text{g/kg}$, making this one of the most sensitive end points for TCDD immunotoxicity. These results have recently been verified for 0.1 $\mu\text{g/kg}$ and extended to show enhanced mortality to influenza virus at 0.01 $\mu\text{g/kg}$ by Burleson et al. (submitted). TCDD treatment of rats significantly augmented influenza virus replication in the lungs. This effect was correlated with a significant suppression of virus-augmented but not spontaneous NK cell activity (Yang et al., submitted).

TCDD exposure also results in more severe parasitic diseases. Tucker et al. (1986) studied the effects of TCDD administration on *Plasmodium yoelii* 17 XNL, a nonlethal strain of malaria, in 6- to 8-week-old B6C3F₁ female mice. A single dose of TCDD at 5 $\mu\text{g/kg}$ or 10 $\mu\text{g/kg}$ per os resulted in increased susceptibility to *P. yoelii*. The peak parasitemia was greater and of longer duration in TCDD-treated animals than in controls, the difference being significant at 5 $\mu\text{g/kg}$ on day 10 and at 10 $\mu\text{g/kg}$ on days 12 and 14. A single dose of TCDD at 10 or 30 $\mu\text{g/kg}$ intraperitoneally 7 days prior to infection of B6C3F₁ mice with *Trichinella spiralis* resulted in delayed onset of adult parasite elimination and at 1.0 $\mu\text{g/kg}$ TCDD suppressed the proliferative response of splenocyte and mesenteric lymph node cells stimulated with *T. spiralis* antigen (Luebke et al., 1994). Tissue levels of TCDD were also higher in infected versus noninfected mice in this study. In a separate study, TCDD at 1, 10, or 30 $\mu\text{g/kg}$ administered intraperitoneally 7 days prior to infection of Fischer 344 rats with *T. spiralis* did not affect adult parasite elimination. Furthermore, proliferative responses of lymphocytes from rats dosed at 30 $\mu\text{g/kg}$ and stimulated with parasite antigen were enhanced in contrast to the suppression observed in B6C3F₁ mice (Luebke et al., submitted).

Luster et al. (1980a) demonstrated enhanced growth of transplanted tumors in mice treated with TCDD at doses of 1.0 or 5.0 $\mu\text{g/kg}$ in B6C3F₁ mice. Mothers were given TCDD by gavage at day 14 of gestation and again on days 1, 7, and 14 following birth; host resistance studies were performed 6 to 8 weeks after weaning. This exposure protocol resulted in an increased incidence of PYB6 tumors in pups from dams receiving repeated doses of 1.0, but not 5.0, $\mu\text{g TCDD/kg}$.

DRAFT--DO NOT QUOTE OR CITE

Although it is clear that TCDD adversely affects numerous host resistance models detailed above, the effects of TCDD on susceptibility to *Listeria monocytogenes* infections are ambiguous. The disparate results may reflect different study designs, including dose, route, single versus multiple administrations, mouse strain, age, or sex. However, it is clear that TCDD, under certain conditions, results in increased susceptibility to *Listeria*. Hinsdill et al. (1980) reported the increased susceptibility of 7-week-old Swiss Webster outbred female mice to *Listeria*. Mice were fed control feed or feed containing 10 or 50 ppb TCDD for 8 weeks, after which they were injected intravenously with 10^5 *Listeria*. Results indicated that the 50-ppb diet increased bacteremia and mortality. Luster et al. (1980a) used doses of 1.0 or 5.0 μg TCDD/kg in B6C3F₁ mice. Mothers were given TCDD by gavage at day 14 of gestation and again on days 1, 7, and 14 following birth, and host resistance studies were performed 6 to 8 weeks after weaning. This exposure protocol resulted in an increased susceptibility to *Listeria* in pups from dams receiving repeated doses of 5.0 μg TCDD/kg. However, Vos et al. (1978) reported that oral administration of 50 μg TCDD/kg once a week for 4 weeks to 3- to 4-week-old male Swiss mice followed by intravenous challenge 4 days after the last dose with *Listeria* had no effect on nonspecific phagocytosis and killing of *Listeria*. House et al. (1990) used B6C3F₁ female mice, 6 to 8 weeks of age and challenged intravenously with *Listeria* 7 to 10 days following a single dose of TCDD at 10, 1.0, and 0.1 $\mu\text{g}/\text{kg}$. TCDD did not enhance mortality to *Listeria*.

In summary, results from host resistance studies provide evidence that exposure to TCDD results in increased susceptibility to bacterial, viral, parasitic, and neoplastic disease. These effects are observed at low doses and likely result from TCDD-induced suppression of immunological function. However, it is interesting that the role of the Ah receptor has not been addressed in terms of host resistance models except in studies on endotoxin hypersensitivity by Rosenthal et al. (1989). Furthermore, the specific immunological functions targeted by TCDD in each of the host resistance models remain to be fully defined.

4.7. IN VITRO IMMUNOTOXIC EFFECTS OF HAH

Investigators in the field of TCDD immunotoxicity have long acknowledged the difficulties in consistently demonstrating the immunotoxicity of TCDD when cells from treated animals are tested *ex vivo* or when TCDD is added to culture *in vitro*. Although effects following *in vitro* and *ex vivo* exposure to TCDD or related HAH on lymphocyte functions have been reported (Tucker et al., 1986; Luster et al., 1988; Dooley and Holsapple, 1988), other laboratories have failed to observe suppression with *in vitro* or *ex vivo* exposure to HAH (Lundberg et al., 1990; Clark et al., 1981; Kerkvliet and Baecher-Steppan, 1988b). In addition, the effects of TCDD seen *in vitro* are sometimes inconsistent with those observed after *in vivo* assessment of immunotoxicity. For example, the rank order of sensitivity to suppression of T helper cell-dependent and T helper cell-independent antibody responses seen *in vivo* (Kerkvliet and Brauner, 1987; Kerkvliet et al., 1990a; House et al., 1990) is not seen *in vitro* (Holsapple et al., 1986a; Tucker et al., 1986), suggesting different cellular targets may be affected following *in vitro* exposure to TCDD. More importantly, some data suggest that suppression of the *in vitro* antibody response may occur independent of the Ah receptor. Tucker et al. (1986) and Holsapple et al. (1986a) reported that direct addition of TCDD *in vitro* suppressed the antibody response to SRBC. However, based on the response of cells from congenic mice as well as a limited structure-activity study, the data of Tucker et al. (1986) supported an Ah receptor-dependent suppression, while the data of Holsapple et al. (1986a) did not. In the latter study, the magnitude of suppression was comparable using cells from responsive B6C3F₁ or congenic heterozygous (B6-Ah^{bd}) mice compared with nonresponsive D2 or homozygous B6-Ah^{dl} mice. In addition, they reported that the 2,7-dichlorodibenzo-*p*-dioxin congener, which lacks affinity for the Ah receptor, was equipotent with TCDD in suppressing the *in vitro* response.

In other studies, Davis and Safe (1991) directly compared the *in vitro* structure-immunotoxicity relationships for a series of HAH congeners that show > 14,900-fold difference in *in vivo* immunotoxic potency. Results of these studies indicated that all of the congeners were equipotent *in vitro* and produced a similar concentration-dependent suppression of the *in vitro* anti-SRBC response using cells from either B6 or D2 mice. Coexposure to the Ah receptor antagonist α -naphthoflavone

DRAFT--DO NOT QUOTE OR CITE

antagonized the immunosuppression induced by either TCDD or 1,3,6,8-TCDF (a weak Ah receptor agonist). Collectively, the results supported a mechanism of suppression in vitro that was independent of the Ah receptor.

The basis for these variable effects of TCDD in vitro are currently not known. However, recent studies by Morris et al. (1991) demonstrated that the in vitro effects of TCDD on the anti-SRBC response were critically dependent on the type and concentration of the serum used in the in vitro culture. Only 3 of 23 lots of serum were able to support a full dose-responsive suppression, and in serum-free cultures, TCDD caused a fifteenfold enhancement of the anti-SRBC response. Thus, differences in media components used in in vitro cultures may account for the different effects seen in vitro in different laboratories. Other factors such as the TCDD carrier/solvent used, the calcium content of the media, or procedures used for preparation of spleen cell suspensions may all contribute to variable effects of TCDD in vitro.

The obvious question relates to the relevance of the in vitro findings to the in vivo immunotoxicity. In this respect, it is important to note that the concentrations of TCDD required for in vitro suppression of immune function ($1-30 \times 10^{-9}$ M) of murine lymphocytes is several orders of magnitude higher than the concentration found in lymphoid tissues following exposure in vivo to an immunotoxic dose of TCDD (Neumann et al., 1992). The amount of TCDD associated with isolated spleen cells obtained from mice 2 days following treatment with $5 \mu\text{g/kg}$ ^3H -TCDD was 2×10^{-15} M per 10^7 spleen cells. Importantly, as much as 50 percent of the radioactivity associated with whole spleen tissue was recovered in the stromal and/or capsular material (i.e., splenic tissue that resisted passage through the mesh screens used for preparation of spleen cell suspensions). These findings suggest that (1) the most potent effects of TCDD on immune function in vivo may be induced indirectly by effects on nonlymphoid cells, or (2) based on the delivered dose of TCDD, this molecule is more toxic than previously thought. Alternatively, TCDD effects in vivo on nonlymphoid cells may amplify the direct effects of TCDD on lymphoid tissue. Certainly, additional studies are needed to elucidate the serum components that are permissive for suppression or enhancement of immune responses in vitro and to determine

their relevance to *in vivo* conditions. Such studies are also likely to provide insight into the mechanisms of TCDD interaction with lymphoid cells.

4.8. INDIRECT MECHANISMS OF HAH IMMUNOTOXICITY

The difficulty in demonstrating consistent, direct effects of TCDD *in vitro* on lymphocytes, the dependence of those effects on serum components, and the requirement for high concentrations of TCDD are all consistent with an indirect mechanism of TCDD on the immune system. One potentially important indirect mechanism is via effects on the endocrine system. Several endocrine hormones have been shown to regulate immune responses, including glucocorticoids, sex steroids, thyroxine, growth hormone, and prolactin. Importantly, TCDD and other HAH have been shown to alter the activity of all of these hormones (see chapter on endocrine system, this document).

Kerkvliet et al. (1990b) reported that exposure of mice to 3,4,5,3',4',5'-HxCB followed by injection of P815 allogeneic tumor cells induced a dose-dependent elevation of serum corticosterone concentrations that correlated with the dose-dependent suppression of the anti-P815 CTL response. However, because adrenalectomy or treatment with the glucocorticoid receptor antagonist RU38486 failed to protect mice from the immunosuppressive effect of HxCB (DeKrey et al., 1990; DeKrey et al., in press), a role for the elevated CS in the suppression of the CTL response seems unlikely. Adrenalectomy and hypophysectomy also failed to prevent TCDD-induced thymic atrophy in rats (van Logten et al., 1980).

Using the P815 allogeneic tumor model, Kerkvliet and Baecher-Steppan (1988a) reported that male mice were more sensitive than female mice to suppression of the CTL response by HxCB (Kerkvliet and Baecher-Steppan, 1988a). Castration of male mice partially ameliorated the immunosuppressive effects of HxCB (DeKrey et al., 1992; DeKrey et al., in press), suggesting a role for testosterone in suppression of the CTL response.

Pazdemik and Rozman (1985) suggest that thyroid hormones may play a role in TCDD immunotoxicity based on the finding that radiothyroidectomy prevented the suppression of the anti-SRBC response in rats treated with TCDD. However, because

thyroidectomy alone suppressed immune function, the significance of the findings requires further study.

4.9. ROLE OF THE THYMUS IN HAH IMMUNOTOXICITY

Thymic involution is one of the hallmarks of exposure to TCDD and related HAH in all species examined. In mice, thymic involution occurs by an Ah receptor-dependent mechanism (Poland and Knutson, 1982). Because the thymus plays a critical role in the ontogeny of T lymphocytes, thymic involution is often referred to as an immunotoxic effect. However, although an intact thymus is crucial to the developing immune system during the prenatal and early postnatal period of rodents as well as during the prenatal period of humans, the physiological role played by the thymus in adult life has not been established. In animal models, adult thymectomy has little effect on the quantity or quality of T lymphocytes, which have already matured and populated the secondary lymphoid organs (Benjamini and Leskowitz, 1991). Likewise, in humans, childhood and adult thymectomy produces no clearly identifiable adverse consequences in terms of altered immune function, although some might argue that such studies have not been done. Based on this knowledge, it is not surprising that a direct relationship between the effects of TCDD on the thymus and immune suppression has not been established in studies using adult animals. In fact, adult thymectomy prior to HAH exposure did not modify TCDD- or HpCDD-induced suppression of the anti-SRBC response (Tucker et al., 1986; Kerkvliet and Brauner, 1987). Furthermore, suppression of immune responses occurs at dose levels of HAH significantly lower than those required to induce thymic atrophy (Vos et al., 1978; Silkworth and Antrim, 1985; Holsapple et al., 1986b; Tucker et al., 1986; Kerkvliet and Brauner, 1990a). Thus, it is clear that thymic involution does not represent a surrogate marker for TCDD immunotoxicity in adult animals. On the other hand, it is possible that chronic exposure to TCDD resulting in a chronic thymic atrophy may produce more delayed, subtle effects on immune function not yet identified (Clarke and MacLennan, 1986).

In contrast to adult animals, congenital thymic aplasia or neonatal thymectomy results in severe reduction in the number and function of T lymphocytes and produces a potentially lethal wasting disease (Benjamini and Leskowitz, 1991). Similarly, there is evidence from

studies carried out in the 1970s that rodents exposed to TCDD or PCBs during the prenatal or neonatal period are more sensitive to immune suppression as compared with rodents exposed as adults and that the prenatal effects are more selective for cell-mediated immunity (Vos and Moore, 1974; Faith and Moore, 1977; Luster et al., 1980a). TCDD has also been shown to alter thymocyte differentiation in vitro in cell cultures (Greenlee et al., 1985; Cook et al., 1987) and organ cultures (Dencker et al., 1985; d'Argy et al., 1989) as well as in vivo following prenatal exposure to TCDD (Blaylock et al., 1992). These observations suggest that altered thymic T cell maturation induced by TCDD in the thymus may play an important role in the suppressed immune function of prenatally exposed animals. However, because TCDD also influences B cell development in the bursa of chick embryos (Nikolaidis et al., 1990) as well as lymphocyte stem cells in the fetal liver and bone marrow of mice (Fine et al., 1989, 1990), other mechanisms of immunotoxicity are also likely to be important.

4.10. IMMUNOTOXICITY FOLLOWING PRENATAL/NEONATAL EXPOSURE TO HAH

The reported increase in susceptibility of very young animals to HAH immunotoxicity necessitates a close examination of the available literature on prenatal or neonatal immunotoxic effects. Several studies have examined immune function in mice, rats, and guinea pigs following exposure to TCDD or PCB during fetal development (Vos et al., 1973; Vos and Moore, 1974; Thomas and Hinsdill, 1979; Luster et al., 1980a).

The results of three major studies in which exposure of the progeny occurred via placental transfer and lactation are summarized in Table 4-5. The most sensitive indicator of TCDD immunotoxicity in these studies was an increase in the growth of transplanted tumor cells in the offspring of B6C3F₁ mice (Ah responsive strain) treated with 1 µg/kg TCDD at 4 weekly intervals. (Total TCDD dose to dam was 4 µg/kg; dose to offspring was not determined.) The offspring of Swiss mice fed a diet containing 1 ppb TCDD for 7 weeks showed enhanced mortality following endotoxin challenge, while the plaque-forming cell response to SRBC and delayed hypersensitivity response were suppressed in offspring of mice fed 5.0-ppb TCDD diets. (Estimated daily dose to 20 g dam consuming 5 g of 5-ppb

DRAFT--DO NOT QUOTE OR CITE

Table 4-5. Immunotoxic Effects of TCDD in the Offspring Following Prenatal/Neonatal Exposure to TCDD

Protocol ^a	End points	Effect	LOAEL [*]
Study #1: Pregnant B6 or B6C3F ₁ mice given 1, 2, 5, or 15 µg/kg TCDD orally on day -7, 0, +7, +14 relative to parturition on day 0	PYB6 tumor incidence	Increased	1 µg/kg × 4
	Allograft rejection time	Increased	2 µg/kg × 4
	Body, thymus, spleen wt	Decreased	5 µg/kg × 4
	Bone marrow cellularity	Decreased	5 µg/kg × 4
	T cell blastogenesis	Decreased	5 µg/kg × 4
	<i>Listeria monocytogenes</i> -induced mortality	Decreased	5 µg/kg × 4
	Bone marrow colony formation (CFU-S)	Decreased	5 µg/kg × 4
	LPS blastogenesis	--	> 15 µg/kg × 4
	Anti-SRBC serum titers	--	> 15 µg/kg × 4
Study #2: Pregnant Swiss mice fed diets containing 1.0, 2.5, or 5.0 ppb TCDD for 7 weeks prenatally and postnatally	Endotoxin mortality	Increased	1.0 ppb diet
	Thymus weight	Decreased	2.5 ppb diet
	PFC response to SRBC	Decreased	5.0 ppb diet
	DTH response	Decreased	5.0 ppb diet
	Anti-SRBC serum titers	--	> 5.0 ppb diet
	T and B cell blastogenesis	--	> 5.0 ppb diet
	<i>Listeria</i> -induced mortality	--	> 5.0 ppb diet
Study #3: Pregnant Fischer 344 rats given 1 or 5 µg/kg TCDD orally on day -3, 0, +7, and +14 relative to parturition on day 0	Allograft rejection time	Increased	5 µg/kg × 4
	T cell blastogenesis	Decreased	5 µg/kg × 4
	DTH response	Decreased	5 µg/kg × 4
	<i>Listeria</i> -induced mortality	Decreased	5 µg/kg × 4
	Body and thymus weight	Decreased	5 µg/kg × 4
	Anti-BGG serum titers	--	> 5 µg/kg × 4

^aStudy #1 (Vos and Moore, 1974; Luster et al., 1980a); Study #2 (Thomas and Hinsdill, 1979); Study #3 (Vos and Moore, 1974; Faith and Moore, 1977).

^{*}Abbreviations used: LOAEL - lowest observable adverse effect level; BGG - bovine gamma globulin; LPS - lipopolysaccharide; PHA - phytohemagglutinin; Con A - Concanavalin A; SRBC - sheep red blood cells; DTH - delayed-type hypersensitivity; PFC - plaque-forming cell; CFU-S - colony-forming units-spleen.

DRAFT--DO NOT QUOTE OR CITE

TCDD diet is equivalent to 1.25 $\mu\text{g/kg}$ TCDD/day.) Rats appeared to be more resistant to the immunotoxic effects of prenatal or neonatal exposure to TCDD based on the finding that 5, but not 1, $\mu\text{g/kg}$ TCDD given four times at weekly intervals produced immunotoxicity in the offspring. Immunotoxic end points that were unaffected by the highest exposure levels in these studies included blastogenesis induced by LPS and serum antibody titers to SRBC and bovine gamma globulin (BGG).

Two recent studies have examined immune function in offspring of female mice exposed to TCDD (Holladay et al., 1991) or PCB (Kanechlor 500) (Takagi et al., 1987) but that were cross-fostered to unexposed lactating mice at birth. Thus, exposure was limited to in utero exposure. (It is important to recognize that rodents are born with an immature immune system that matures in the first few weeks following birth. In contrast, the human immune system is considered to be more mature at birth.) B6 mice exposed to 3.0 $\mu\text{g/kg}$ TCDD on gestational days 6 to 14 gave birth to offspring that had significant thymic atrophy and hypoplasia measured on gestational day 18 or on day 6 postnatally. The thymic effects were no longer apparent by day 14. At 7 to 8 weeks postnatally, mitogen responses and antibody plaque-forming cell response to SRBC were unaltered, while the CTL response was significantly suppressed compared with controls (Holladay et al., 1991). These results suggest a selectivity of prenatal TCDD on the CTL and not the T helper cells involved in the antibody response to SRBC. In contrast to these results, Takagi et al. (1987) exposed female C3H mice per os to 50 mg/kg Kanechlor 500 twice per week for 4 weeks, at which time steady-state tissue levels were noted. The offspring derived from mating to unexposed males had an unaltered antibody response to the T-independent antigen DNP-dextran. On the other hand, carrier-primed T helper cell activity assessed by adoptive transfer was significantly suppressed by PCB exposure when assessed 4 and 7 weeks after birth but fully recovered by 11 weeks. Together, these studies confirm prior studies to indicate that T cell function is selectively altered by HAH when exposure is prenatal. Although both T helper cells and CTL show altered function, T helper cell activity may recover faster than CTL function.

Fine et al. (1990) reported on TCDD levels in offspring following maternal treatment with TCDD (10 $\mu\text{g/kg}$) on gestational day 14. The fetal liver had the highest concentration on gestational day 18 (235 fg/mg), which declined slightly by postnatal day 6 to around 100

fg/mg. The concentration of TCDD in the thymus on gestational day 18 was 140 fg/mg, which declined to 20 fg/mg on day 6 after birth. (These thymic TCDD concentrations are equivalent to 60 to 425 pM, assuming 1 kg of tissue is equivalent to 1 liter of water.) TCDD concentrations in the spleen remained constant at about 40 fg/mg during the same time frame, while bone marrow concentrations were very low (about 3 fg/mg). These concentrations of TCDD were associated with thymic atrophy (Fine et al., 1989) and significant reduction in the ability of prothymocytes in liver and bone marrow to repopulate an irradiated thymus (Fine et al., 1990).

4.11. IMMUNOTOXICITY OF HAH IN NONHUMAN PRIMATES

A limited number of studies using nonhuman primates as surrogate models for humans have been conducted to assess HAH immunotoxicity. Immunological effects were described in rhesus monkeys and their offspring chronically exposed to TCDD at levels of 5 or 25 ppt for 4 years (Hong et al., 1989). In the mothers, the total number of T cells increased in monkeys fed 25 ppt TCDD, with a selective increase in CD8⁺ cells and a decrease in CD4⁺ cells. However, no significant effect on T cell function was established when assessed as proliferation response to mitogens, alloantigens, or xenoantigens. NK cell activity and production of antibodies to tetanus immunization were normal. In the offspring of TCDD-exposed dams examined 4 years after exposure, a significantly increased antibody response to tetanus toxoid immunization was observed that correlated with TCDD tissue levels. The body burden of TCDD in the offspring ranged from a low of 290 ppt to a high of 1,400 ppt. Interestingly, there was no strict correlation between exposure levels and resulting body burden.

In other TCDD studies, a single injection of TCDD in marmosets (*Callithrix jacchus*) resulted in a delayed decrease in the percentage of CD4⁺ T cells and CD20⁺ B cells in the blood and an increase in the percentage of CD8⁺ cells (Neubert et al., 1990). The total number of T cells was not significantly altered by TCDD exposure. The CD4⁺ subset most affected was the CDw29⁺ "helper-inducer" or "memory" subset, with significant effects observed after a TCDD dose of 10 ng/kg. The no-observed-effect level (NOEL) for this effect was 3 ng/kg TCDD. Concomitant with suppression of the CDw29 subset in TCDD-

DRAFT--DO NOT QUOTE OR CITE

treated animals, the percentage of CD4⁺CD45RA⁺ cells increased. This subset has been classified as "suppressor-inducer" or "naive" cells. The changes in the T cell subsets were intensified following in vitro culture of the cells with mitogen (Neubert et al., 1991). Interestingly, however, a recent study from the same laboratory reported that chronic exposure of young marmosets to very low levels of TCDD (0.3 ng/kg/week for 24 weeks) produced the opposite effect on the CD4⁺CDw29⁺ subset, resulting in a significant increase in this population (Neubert et al., 1992). Concomitantly, the CD4⁺CD45RA⁺ subset decreased. Upon transfer of the animals to a higher dose of TCDD (1.5 ng/kg/week) for 3 weeks, the enhancement effect was reversed, and suppression of the CD4⁺CDw29⁺ subset was observed, with maximum suppression after 6 weeks of exposure to the higher dose. In addition, the CD8⁺CD56⁺ T cytotoxic T cell subset was transiently increased but normalized even though TCDD dosing continued. After discontinuation of dosing, the reduction in the percentage and absolute number of CD4⁺CDw29⁺ cells persisted for 5 weeks, reaching normal range 7 weeks later. These results led the authors to conclude that "extrapolations of the results obtained at higher doses to very low exposures is not justified with respect to the effects induced by TCDD on the immune system of marmosets."

The immunomodulatory effects of chronic low-level PCB exposure in monkeys has also been investigated. In early studies, Thomas and Hinsdill (1978) reported that rhesus monkeys fed diets containing 2.5 or 5 mg/kg of Aroclor 1248 had significantly suppressed antibody response to SRBC but not to tetanus toxoid (TT). These monkeys also had chloracne, alopecia, and facial edema. Similarly, exposure of cynomolgus monkeys to Aroclor 1254 (100 or 400 µg/kg/day) for 3 months suppressed antibody responses to SRBC but not TT (Truelove et al., 1982). Suppressive effects on anti-SRBC responses were more severe in cynomolgus monkeys when the PCB mixture contained PCDFs (Hori et al., 1982). Tryphonas et al. (1989; 1991a,b) have recently reported results of studies in rhesus monkeys exposed chronically to Aroclor 1254 (5-80 µg/kg/day) for 23 or 55 months. These exposures resulted in steady-state blood PCB levels that ranged from a mean low of 0.01 ± 0.001 ppm in the 5 µg/kg group to a mean high of 0.11 ± 0.01 ppm in the 80 µg/kg group. The only consistently altered immune parameter was the primary and anamnestic antibody responses to SRBC, which were suppressed in a dose-dependent manner. In contrast, the antibody

DRAFT--DO NOT QUOTE OR CITE

response to pneumococcus vaccine antigen measured at 55 months of exposure was not significantly altered. At 23 months, the percentage of T helper cells in the blood was significantly decreased in the 80 $\mu\text{g}/\text{kg}$ group, and the percentage and absolute number of T suppressor cells was increased; however, these effects were not apparent at 55 months of exposure (Tryphonas et al., 1991b). Lymphoproliferative responses to PHA and Con A were not significantly altered at 23 months but were dose dependently suppressed at 55 months. Proliferation to alloantigens was not significantly altered. Likewise, serum immunoglobulin and hydrocortisone levels did not differ between treatment groups. After 55 months, the chemiluminescent response (time to peak) of monocytes was slower in PCB-exposed cells. Also noted at 55 months was a significant elevation in serum hemolytic complement levels, a dose-related increase in NK cell activity, and a dose-related increase in thymosin alpha-1 levels but not thymosin beta-4 levels (Tryphonas et al., 1991a). Effects on interferon levels were inconsistent, and TNF production was not altered.

The studies in nonhuman primates are important from the standpoint that the antibody response to SRBC emerges as the only immunological parameter consistently suppressed by HAH in several different animal species. A notable exception is the recent report that TCDD does not suppress the antibody response to SRBC in rats (Smialowicz et al., 1994). Other immunological end points such as total T cell numbers, percentages of T cell subsets, lymphoproliferative responses, and DTH responses are inconsistently increased or decreased in various studies. At the present time, it is not clear why the antibody response to SRBC is most consistently altered by HAH exposure in many different species. The sensitivity of the anti-SRBC response does not appear to be due solely to the T cell dependency of the response because antibody responses to other T-dependent antigens (e.g., TT, BGG) are not suppressed and may be enhanced following HAH exposure. It is possible that the particulate nature of the SRBC antigens is an important factor even though a mechanistic basis for this is not readily apparent. The sensitivity of the technique used to quantify the antibody response may also contribute to apparent increased sensitivity of the SRBC model, which is most often measured as the PFC response rather than serum antibody titers that are usually more variable. Nonetheless, the finding that the SRBC response is also suppressed in nonhuman

primates exposed to PCB lends support to the use of the anti-SRBC data generated in mice to calculate TEFs for immunotoxicity.

4.12. IMMUNOTOXICITY OF HAH IN HUMANS

The immunotoxicity of TCDD and related HAH in humans has been the subject of several studies derived from accidental and occupational exposures to PCBs, PBBs, and TCDD. Immunological assessment was carried out on patients who consumed acnegenic and hepatotoxic doses of PCDF- and PCB-contaminated rice oil in Taiwan in 1979. Clinical symptoms were primarily related to increased frequency of various kinds of infection, especially of the respiratory tract and skin (Lu and Wu, 1985). Immunologic effects included decreased serum IgA and IgM, but not IgG, decreased percentage of T cells in blood related to decreased CD4⁺ T helper cells and increased CD8⁺ T suppressor cells, and suppressed dermal delayed-type hypersensitivity responses to streptokinase-streptodornase and tuberculin antigens (reviewed by Lu and Wu, 1985). The percentage of anergic patients increased, and the degree of induration decreased with increased PCB concentration in the blood. In contrast, lymphoproliferative responses of PBL to PHA, pokeweed mitogen (PWM), and tuberculin, but not Con A, were significantly augmented in PCB-exposed patients. PCB concentrations in the blood ranged from 3 to 1,156 ppb, with a mean of 89 ± 6.9 ppb. The oil was contaminated at PCB concentrations of 4.8 to 204.9 ppm, with a mean of 52 ± 39 ppm.

Immunotoxic effects were also described in Michigan dairy farmers exposed to PBBs via contaminated dairy products and meat in 1973 (Bekesi et al., 1979). Like PCB-exposed patients, the percentage and absolute numbers of T cells in peripheral blood of PBB-exposed farmers were significantly reduced compared with a control group. However, in contrast to PCB, lymphoproliferation responses to PHA, PWM, and allogeneic leukocytes were significantly decreased in PBB-exposed persons. Also in contrast to PCB, skin testing using standard recall antigens indicated that PBB-exposed Michigan dairy farmers had significantly increased responses, particularly to candida and Varidase. Tissue levels of PBB in the subjects were not determined in these studies.

Webb et al. (1989) reported the findings from immunologic assessment of 41 persons from Missouri with documented adipose tissue levels of TCDD resulting from occupational, recreational, or residential exposure. Of the participants, 16 had tissue TCDD levels less than 20 ppt; 13 had levels between 20 and 60 ppt; and 12 had levels greater than 60 ppt. The highest level was 750 ppt. Data were analyzed by multiple regression based on adipose tissue level and the clinical dependent variable. Increased TCDD levels were correlated with an increased percentage and total number of T lymphocytes. CD8⁺ and T11⁺ T cells accounted for the increase, while CD4⁺ T cells were not altered in percent or number. Lymphoproliferative responses to Con A, PHA, PWM, or TT were unaltered, as was the cytotoxic T cell response. Serum IgA, but not IgG, was increased. No adverse clinical disease was associated with TCDD levels in these subjects. Only 2 of the 41 subjects reported a history of chloracne. These findings differ from those reported for the Quail Run Mobile Home Park residents (tissue levels unknown) in which decreased T cell numbers (T3, CD4, and T11) and suppressed cell-mediated immunity were reported (Hoffman et al., 1986). However, subsequent retesting of these anergic subjects failed to confirm the anergy (Evans et al., 1988). On the other hand, when serum from some of these individuals was tested for levels of the thymic peptide, thymosin alpha-1, the entire frequency distribution for the TCDD-exposed group was shifted toward lower thymosin alpha-1 levels (Stehr-Green et al., 1989). The statistically significant difference between the TCDD-exposed persons and controls remained after controlling for age, sex, and socioeconomic status, with a trend of decreasing thymosin alpha-1 levels with increasing number of years of residence in the TCDD-contaminated residential area. The thymosin alpha-1 levels were not correlated with changes in other immune system parameters nor with any increased incidence of clinically diagnosed immune suppression. The decrease in thymosin alpha-1 levels in humans contrasts with the increase in thymosin alpha-1 seen in PCB-treated monkeys (Tryphonas et al., 1991b). Finally, Mocarelli et al. (1986) reported studies on the immune status of 44 children, 20 of whom had chloracne, that were exposed to TCDD following an explosion at a herbicide factory in Seveso, Italy. No abnormalities were found in the following parameters: serum immunoglobulin concentrations, levels of circulating complement, or lymphoproliferative responses to T and B cell mitogens. Interestingly, in a study conducted

DRAFT--DO NOT QUOTE OR CITE

6 years after the explosion, a different cohort of TCDD-exposed children exhibited a significant increase in complement protein levels, which correlated with the incidence of chloracne as well as increased numbers of peripheral blood lymphocytes and increased lymphoproliferative responses (Tognoni and Bonaccorsi, 1982). However, no specific health problems were correlated with dioxin exposure in these children.

It is readily apparent that no clear pattern of immunotoxicity to HAH emerges from these studies in humans. In some cases, T cell numbers increase; in others, they decrease. The findings are not unlike the varied and often conflicting reports found in the literature regarding animal studies of HAH immunotoxicity. The basis for the lack of consistent, significant exposure-related effects is unknown and may be dependent on several factors. Most notable in this regard are the generic difficulties in assessing subclinical immunomodulation, particularly in outbred human populations. Most immunological assays have a very broad range of normal responses reducing the sensitivity to detect small changes. Similarly, the assays used to examine immune function in humans exposed to TCDD and related HAH have unfortunately been based to a greater extent on what was clinically "doable" (e.g., mitogen responsiveness) rather than on assays that have been shown to be sensitive to TCDD in animal studies. Thus, the lack of consistent or significant immunotoxic effects in humans resulting from TCDD exposure may be as much a function of the assays used as the immune status of the cohort. In addition, few studies have examined the immune status of individuals with known, documented exposure to HAH. Rather, cohorts based on presumption of exposure have been studied. There is some evidence to suggest that the lack of significant effects may sometimes be due to the inclusion of subjects that had little or no actual exposure to TCDD (Webb et al., 1989). Likewise, the important role that Ah phenotype plays in TCDD immunotoxicity has not been considered when addressing human sensitivity. Whether there are human equivalents of murine Ah^{bb} and Ah^{dd} types is not known. Finally, in most studies, the assessment of immune function in exposed populations was carried out long after exposure to TCDD ceased. Thus, recovery from the immunotoxic effects of TCDD may have occurred.

Recently, several laboratories have examined the direct effects of TCDD on human lymphocytes. Neubert et al. (1991) reported decreased peripheral blood lymphocyte (PBL)

subpopulations from humans and nonhuman primates cultured in the presence of TCDD. CD4⁺CD29⁺ helper-inducer/memory T cells and CD20⁺ B cells were dose dependently decreased in PWM-stimulated cultures of human PBLs at concentrations as low as 10⁻¹²-10⁻¹⁴ M TCDD. However, an attempt to corroborate these findings failed to detect any suppression in human PBL subpopulations, including CD4⁺CD29⁺ helper-inducer/memory T cells and CD19⁺ B cells, at TCDD concentrations ranging from 10⁻⁷ to 10⁻¹⁴ M (Lang et al., 1994). Furthermore, PWM- or anti-CD3-stimulated lymphocyte proliferation was not altered by TCDD at concentrations of 10⁻⁷ to 10⁻¹¹ M (Lang et al., 1994). Similar results were obtained by Wood et al. (1992) who reported that exposure of human tonsillar lymphocytes (HTLs) to concentrations of 3 × 10⁻⁸ to 3 × 10⁻¹⁰ M TCDD did not affect either PWM-induced proliferation or IgM antibody production. In contrast, 3 × 10⁻⁸ to 3 × 10⁻¹⁰ M TCDD suppressed toxic shock syndrome toxin (TSST-1)-induced IgM secretion of HTL B cells (Wood et al., 1993a). The sensitivity of the HTL B cells to TCDD suppression of TSST-1-induced IgM secretion, however, was found to be highly variable among the different donors. In a separate study, concentrations of 3 × 10⁻⁸ to 3 × 10⁻¹⁰ M TCDD suppressed the background proliferation and IgM secretion of low-density, but not high-density, HTL B cells (Wood et al., 1993b). TCDD also suppressed LPS plus T cell replacement factor-stimulated proliferation and IgG secretion of low-density, but not high-density, HTL B cells. These results suggest that TCDD may have a direct effect on HTL low-density B cells and that this lymphocyte subpopulation may be a sensitive target for TCDD. Further work is necessary to corroborate these findings.

In summary, based on animal data, one might speculate that any future study to determine HAH immunotoxicity in humans should evaluate their antibody response to SRBC. However, it **should be emphasized** that even the relatively low exposure levels that have been shown to **suppress the anti-SRBC response** in nonhuman primates resulted in blood and tissue PCB or TCDD levels that far exceed the levels measured in humans in most studies published to date involving environmental exposure. Thus, even the anti-SRBC response may not have been sensitive enough to demonstrate immune suppression in these cohorts. Given the current lack of data correlating clinical immunological end points with immune status in humans (except in cases of overt immune deficiencies), massive retrospective studies

AG:
Wood et al.

of poorly defined exposure groups cannot be justified to try to "prove" that immune modulation has occurred in these people. Rather, such efforts would be better directed toward the establishment of a broad database of normal values for the clinical immunology end points that may be of use in immunotoxicity assessments. In conjunction with this effort, research must focus on the definition of sensitive end points (i.e., biomarkers) of immune dysfunction in humans so that, in the future, emergency response teams could respond rapidly to accidental exposures to assess the immunological status of the exposed persons. To validate these biomarkers, there is a parallel need for animal research to identify TCDD-sensitive immune end points in animals that can also be measured in humans to establish correlative changes in the biomarker and immune function. In particular, it will be important to determine in animal models how well changes in immune function in the lymphoid organs (e.g., spleen, lymph nodes) correlate with changes in the expression of lymphocyte subset/activation markers in peripheral blood. Until such correlations are established, the interpretation of changes observed in subsets or activation markers in human peripheral blood lymphocytes in terms of health risk will be limited to speculation. Research must also continue to develop well-defined animal models using multiple animal species that will lead to an understanding of the underlying mechanisms of HAH immunotoxicity. For example, there is a clear need to document Ah receptor involvement in the immunotoxicity of TCDD and related HAH in species other than mice. These studies need to go beyond descriptive immunotoxicity assessment to determine the mechanistic basis for differences in species sensitivity to TCDD immunotoxicity following both acute and chronic exposure. In the interim, the available database derived from well-controlled animal studies on HAH immunotoxicity can be used for establishment of no-effect levels and acceptable exposure levels for human risk assessment of TCDD using the same procedures that are used for other noncarcinogenic toxic end points. Because the antibody response to SRBC has been shown to be dose dependently suppressed by TCDD and related HAH in several animal species, this database is best suited for current application to risk assessment.

REFERENCES

- Ackermann, M. F.; Gasiewicz, T. A.; Lamm, K. R.; Germolec, D. R.; Luster, M. I. (1989) Selective inhibition of polymorphonuclear neutrophil activity by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Appl. Pharmacol.* 101: 470-480.
- Alsharif, N. Z.; Lawson, T.; Stohs, S. J. (1990) TCDD-induced production of superoxide anion and DNA single strand breaks in peritoneal macrophages of rats. *The Toxicologist* 10: 276 (Abs. 1102).
- Bekesi, J. G.; Anderson, H. A.; Roboz, J. P.; Roboz, J.; Fischbein, A.; Selikoff, I. J.; Holland, J. F. (1979) Immunologic dysfunction among PBB-exposed Michigan dairy farmers. *Ann. N. Y. Acad. Sci.* 320: 717-728.
- Benjamini, E.; Leskowitz, S. (1991) *Immunology. A short course.* 2d ed. New York, NY: Wiley-Liss, Inc.; p. 26.
- Biegel, L.; Harris, M.; Davis, D.; Rosengren, R.; Safe, L.; Safe, S. (1989) 2,2',4,4',5,5'-Hexachlorobiphenyl as a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin antagonist in C57Bl/6 mice. *Toxicol. Appl. Pharmacol.* 97: 561-571.
- Birnbaum, L. S.; McDonald, M. M.; Blair, P. C.; Clark, A. M.; Harris, M. W. (1990) Differential toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in C57Bl/6 mice congenic at the Ah locus. *Fundam. Appl. Toxicol.* 15: 186-200.
- Blaylock, B. L.; Holladay, S. D.; Comment, C. E.; Heindel, J. J.; Luster, M. I. (1992) Exposure to tetrachlorodibenzo-*p*-dioxin (TCDD) alters fetal thymocyte maturation. *Toxicol. Appl. Pharmacol.* 112: 207-213.
- Braley-Mullen, H. (1982) Differential effect of activated T amplifier cells on B cells responding to thymus-independent type-1 and type-2 antigens. *J. Immunol.* 129: 484-489.
- Burleson, G. R.; Lebrech, H.; Yang, Y. G.; Ibanes, J. D.; Pennington, K. N. Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on influenza virus host resistance in mice (submitted).
- Clark, D. A.; Gauldie, J.; Szewczuk, M. R.; Sweeney, G. (1981) Enhanced suppressor cell activity as a mechanism of immunosuppression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Proc. Exp. Biol. Med.* 168: 290-299.

DRAFT--DO NOT QUOTE OR CITE

- Clark, D. A.; Sweeney, G.; Safe, S.; Hancock, E.; Kilburn, D. G.; Gauldie, J. (1983) Cellular and genetic basis for suppression of cytotoxic T cell generation by haloaromatic hydrocarbons. *Immunopharmacology* 6: 143-153.
- Clark, G. C.; Blank, J. A.; Germolec, D. R.; Luster, M. I. (1991a) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin stimulation of tyrosine phosphorylation in B lymphocytes: potential role in immunosuppression. *Mol. Pharmacol.* 39: 495-501.
- Clark, G. C.; Taylor, M. J.; Tritscher, A. M.; Lucier, G. W. (1991b) Tumor necrosis factor involvement in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated endotoxin hypersensitivity in C57Bl/6 mice congenic at the Ah locus. *Toxicol. Appl. Pharmacol.* 111: 422-431.
- Clarke, A. G.; MacLennan, K. A. (1986) The many facets of thymic involution. *Immunol. Today* 7: 204-205.
- Cook, J. C.; Dold, K. M.; Greenlee, W. F. (1987) An in vitro model for studying the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to human thymus. *Toxicol. Appl. Pharmacol.* 89: 256-268.
- Cuthill, S.; Wilhelmsson, A.; Mason, G. G. F.; Gillner, M.; Poellinger, L.; Gustafsson, J.-A. (1988) The dioxin receptor: a comparison with the glucocorticoid receptor. *J. Steroid Biochem.* 30: 277-280.
- d'Argy, R.; Bergman, J.; Dencker, L. (1989) Effects of immunosuppressive chemicals on lymphoid development in fetal thymus organ cultures. *Pharmacol. Toxicol.* 64: 33-38.
- Davis, D.; Safe, S. (1988) Immunosuppressive activities of polychlorinated dibenzofuran congeners: quantitative structure-activity relationships and interactive effects. *Toxicol. Appl. Pharmacol.* 94: 141-149.
- Davis, D.; Safe, S. (1989) Dose-response immunotoxicities of commercial polychlorinated biphenyls (PCBs) and their interactions with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Lett.* 48: 35-43.
- Davis, D.; Safe, S. (1990) Immunosuppressive activities of polychlorinated biphenyls in C57Bl/6N mice: structure-activity relationships as Ah receptor agonists and partial antagonists. *Toxicology* 63: 97-111.

DRAFT--DO NOT QUOTE OR CITE

- Davis, D.; Safe, S. (1991) Halogenated aryl hydrocarbon-induced suppression of the in vitro plaque-forming cell response to sheep red blood cells is not dependent on the Ah receptor. *Immunopharmacology* 21: 183-190.
- DeKrey, G. K.; Steppan, L. B.; Deyo, J. A.; Kerkvliet, N. I. (1990) Adrenalectomy (ADX) and 3,4,5,3',4',5'-hexachlorobiphenyl (HxCB) suppression of cytotoxic T lymphocyte (CTL) response to P815 allogeneic tumor in C57Bl/6 mice. *The Toxicologist* 10: 290 (Abs. 1157).
- DeKrey, G. K.; Deyo, J. A.; Kerkvliet, N. I. (1992) Castration (ODX) partially alleviates the suppression of cytotoxic T lymphocyte (CTL) activity by 3,3',4,4',5,5'-hexachlorobiphenyl (HxCB). *The Toxicologist* 12: 132 (Abs. 442).
- DeKrey, G. K.; Baecher-Steppan, L.; Deyo, J. A.; Smith, B. B.; Kerkvliet, N. I. PCB-induced immune suppression: castration but not adrenalectomy or RU 38486 treatment partially restores the suppressed cytotoxic T lymphocyte response to alloantigen. *J. Pharmacol. Exp. Ther.* (in press).
- Dencker, L.; Hassoun, E.; d'Argy, R.; Alm, G. (1985) Fetal thymus organ culture as an in vitro model for the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and its congeners. *Mol. Pharmacol.* 28: 357-363.
- Dooley, R. K.; Holsapple, M. P. (1988) Elucidation of cellular targets responsible for tetrachlorodibenzo-*p*-dioxin (TCDD)-induced suppression of antibody responses: the role of the B lymphocyte. *Immunopharmacology* 16: 167-180.
- Dooley, R. K.; Morris, D. L.; Holsapple, M. P. (1990) Elucidation of cellular targets responsible for tetrachlorodibenzo-*p*-dioxin (TCDD)-induced suppression of antibody response. 2. Role of the T lymphocyte. *Immunopharmacology* 19: 47-58.
- Evans, R. G.; Webb, K. B.; Knutsen, A. P.; Roodman, S. T.; Robers, D. W.; Bagby, J. R.; Garrett, W. A.; Andrews, J. S. (1988) A medical follow-up of the health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Arch. Environ. Health* 43: 273-278.
- Faith, R. E.; Moore, J. A. (1977) Impairment of thymus-dependent immune function by exposure of the developing immune system to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *J. Toxicol. Environ. Health* 3: 451-464.

DRAFT--DO NOT QUOTE OR CITE

- Fine, J. S.; Gasiewicz, T. A.; Silverstone, A. E. (1989) Lymphocyte stem cell alterations following perinatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Mol. Pharmacol.* 35: 18-25.
- Fine, J. S.; Gasiewicz, T. A.; Fiore, N. C.; Silverstone, A. E. (1990) Prothymocyte activity is reduced by perinatal 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure. *J. Exp. Pharmacol. Ther.* 255: 1-5.
- Fraker, P. J. (1980) The antibody mediated and delayed type hypersensitivity response of mice exposed to polybrominated biphenyls. *Toxicol. Appl. Pharmacol.* 53: 1-7.
- Greenlee, W. F.; Dold, K. M.; Irons, R. D.; Osborne, R. (1985) Evidence for direct action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on thymic epithelium. *Toxicol. Appl. Pharmacol.* 79: 112-120.
- Hanson, C. D.; Smialowicz, R. J. (1994) Evaluation of the effect of low-level 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure on cell mediated immunity. *Toxicology* (in press).
- Hinsdill, R. D.; Couch, D. L.; Speirs, R. S. (1980) Immunosuppression in mice induced by dioxin (TCDD) in feed. *J. Environ. Pathol. Toxicol.* 4(2-3): 401-425.
- Hoffman, R. E.; Stehr-Green, P. A.; Webb, K. B.; Evans, R. G.; Knutsen, A. P.; Schramm, W. R. F.; Staake, J. L.; Gibson, B. B.; Steinberg, K. K. (1986) Health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *JAMA* 255: 2031-2038.
- Hoglen, N.; Swim, A.; Robertson, L.; Shedlofsky, S. (1992) Effects of xenobiotics on serum tumor necrosis factor (TNF) and interleukin-6 (IL-6) release after LPS in rats. *The Toxicologist* 12: 290 (Abs. 1118).
- Holladay, S. D.; Lindstrom, P.; Blaylock, B. L.; Comment, C. E.; Germolec, D. R.; Heindell, J. J.; Luster, M. I. (1991) Perinatal thymocyte antigen expression and postnatal immune development altered by gestational exposure to tetrachlorodibenzo-*p*-dioxin (TCDD). *Teratology* 44: 385-393.
- Holsapple, M. P.; Dooley, R. K.; McNerney, P. J.; McCay, J. A. (1986a) Direct suppression of antibody responses by chlorinated dibenzodioxins in cultured spleen cells from (C57Bl/6 × C3H)F1 and DBA/2 mice. *Immunopharmacology* 12: 175-186.

DRAFT--DO NOT QUOTE OR CITE

- Holsapple, M. P.; McCay, J. A.; Barnes, D. W. (1986b) Immunosuppression without liver induction by subchronic exposure to 2,7-dichlorodibenzo-*p*-dioxin in adult female B6C3F₁ mice. *Toxicol. Appl. Pharmacol.* 83: 445-455.
- Holsapple, M. P.; Morris, D. L.; Wood, S. C.; Snyder, N. K. (1991a) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin-induced changes in immunocompetence: possible mechanisms. *Annu. Rev. Pharmacol. Toxicol.* 31: 73-100.
- Holsapple, M. P.; Snyder, N. K.; Wood, S. C.; Morris, D. L. (1991b) A review of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin-induced changes in immunocompetence: 1991 update. *Toxicology* 69(3): 219-255.
- Hong, R.; Taylor, K.; Abonour, R. (1989) Immune abnormalities associated with chronic TCDD exposure in rhesus. *Chemosphere* 18: 313-320.
- Hori, S.; Obana, H.; Kashimoto, T.; Otake, T.; Mishimura, H.; Ikegami, N.; Kunita, N.; Uda, H. (1982) Effect of polychlorinated biphenyls and polychlorinated quaterphenyls in cynomolgus monkey (*Macaca fascicularis*). *Toxicology* 24: 123-139.
- House, R. V.; Lauer, L. D.; Murray, M. J.; Thomas, P. T.; Ehrlich, J. P.; Burleson, G. R.; Dean, J. H. (1990) Examination of immune parameters and host resistance mechanisms in B6C3F₁ mice following adult exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Toxicol. Environ. Health* 31: 203-215.
- Jelinek, D. F.; Lipsky, P. E. (1987) Regulation of human B lymphocyte activation, proliferation, and differentiation. *Adv. Immunol.* 40: 1-59.
- Katz, L. B.; Theobald, H. M.; Bookstaff, R. C.; Peterson, R. E. (1984) Characterization of the enhanced paw edema response to carrageenan and dextran in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated rats. *J. Pharmacol. Exp. Ther.* 230: 670-677.
- Kerkvliet, N. I. (1984) Halogenated aromatic hydrocarbons (HAH) as immunotoxicants. In: Kende, M.; Gainer, J.; Chirigos, M., eds. *Chemical Regulation of Immunity in Veterinary Medicine*. New York, NY: Alan R. Liss, Inc.; pp. 369-387. (Progress in clinical and biological research: v. 161).
- Kerkvliet, N. I.; Baecher-Steppan, L. (1988a) Suppression of allograft immunity by 3,4,5,3',4',5'-hexachlorobiphenyl. I. Effects of exposure on tumor rejection and cytotoxic T cell activity. *Immunopharmacology* 16: 1-12.

DRAFT--DO NOT QUOTE OR CITE

- Kerkvliet, N. I.; Baecher-Steppan, L. (1988b) Suppression of allograft immunity by 3,4,5,3',4',5'-hexachlorobiphenyl. II. Effects of exposure on mixed lymphocyte reactivity in vitro and induction of suppressor cells. *Immunopharmacology* 16: 13-23.
- Kerkvliet, N. I.; Brauner, J. A. (1987) Mechanisms of 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD)-induced humoral immune suppression: evidence of primary defect in T cell regulation. *Toxicol. Appl. Pharmacol.* 87: 18-31.
- Kerkvliet, N. I.; Brauner, J. A. (1990a) Flow cytometric analysis of lymphocyte subpopulations in the spleen and thymus of mice exposed to an acute immunosuppressive dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Environ. Res.* 52: 146-164.
- Kerkvliet, N. I.; Brauner, J. A. (1990b) Functional analysis of antigen-presenting cells following antigen challenge: influence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). 29th Annual Meeting of the Society of Toxicology, Miami Beach, Florida; February 12-16, 1990. *The Toxicologist* 10: 289 (Abs. 1155).
- Kerkvliet, N. I.; Oughton, J. A. (1993) Acute inflammatory response to sheep red blood cell challenge in mice treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD): phenotypic and functional analysis of peritoneal exudate cells. *Toxicol. Appl. Pharmacol.* 119: 248-257.
- Kerkvliet, N. I.; Brauner, J. A.; Matlock, J. P. (1985) Humoral immunotoxicity of polychlorinated diphenyl ethers, phenoxyphenols, dioxins and furans present as contaminants of technical grade pentachlorophenol. *Toxicology* 36: 307-324.
- Kerkvliet, N. I.; Steppan, L. B.; Brauner, J. A.; Deyo, J. A.; Henderson, M. C.; Tomar, R. S.; Buhler, D. R. (1990a) Influence of the Ah locus on the humoral immunotoxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) immunotoxicity: evidence for Ah receptor-dependent and Ah receptor-independent mechanisms of immunosuppression. *Toxicol. Appl. Pharmacol.* 105: 26-36.
- Kerkvliet, N. I.; Steppan, L. B.; Smith, B. B.; Youngberg, J. A.; Henderson, M. C.; Buhler, D. R. (1990b) Role of the Ah locus in suppression of cytotoxic T lymphocyte (CTL) activity by halogenated aromatic hydrocarbons (PCBs and TCDD): structure-activity relationships and effects in C57Bl/6 mice. *Fundam. Appl. Toxicol.* 14: 532-541.

DRAFT--DO NOT QUOTE OR CITE

- Kramer, C. M.; Johnson, K. W.; Dooley, R. K.; Holsapple, M. P. (1987) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) enhances antibody production and protein kinase activity in murine B cells. *Biochem. Biophys. Res. Commun.* 145: 25-32.
- Lang, D. S.; Becker, S.; Clark, G. C.; Devlin, R. B.; Koren, H. S. (1994) Lack of direct immunosuppressive effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on human peripheral blood lymphocyte subsets in vitro. *Arch. Toxicol.* (in press).
- Loose, L. D.; Silkworth, J. B.; Mudzinski, S. P.; Pittman, K. A.; Benitz, K. F.; Mueller, W. (1979) Environmental chemical-induced immune dysfunction. *Ecotoxicol. Environ. Safety* 2: 173-198.
- Lu, Y.-C.; Wu, Y.-C. (1985) Clinical findings and immunological abnormalities in Yu-Cheng patients. *Environ. Health Perspect.* 59: 17-29.
- Lubet, R. A.; Lemaire, B. N.; Avery, D.; Kouri, R. E. (1986) Induction of immunotoxicity in mice by halogenated biphenyls. *Arch. Toxicol.* 59: 51-77.
- Luebke, R. W.; Copeland, C. B.; Diliberto, J. J.; Akubue, P. I.; Andrews, D. L.; Riddle, M. M.; Williams, W. C.; Birnbaum, L. S. (1994) Assessment of host resistance to *Trichinella spiralis* in mice following pre-infection exposure to 2,3,7,8-TCDD. *Toxicol. Appl. Pharmacol.* 125 (in press).
- Luebke, R. W.; Copeland, C. B.; Andrews, D. L. Host resistance to *T. spiralis* infection in rats exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (submitted).
- Lundberg, K.; Dencker, L.; Gronvik, K.-O. (1990) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) treatment in vivo on thymocyte functions in mice after activation in vitro. *Int. J. Immunopharmacol.* 12: 459-466.
- Luster, M. I.; Boorman, G. A.; Dean, J. H.; Harris, M. W.; Luebke, R. W.; Padarathsingh, M. L.; Moore, J. A. (1980a) Examination of bone marrow, immunologic parameters and host susceptibility following pre- and postnatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Int. J. Immunopharm.* 2: 301-310.
- Luster, M. I.; Boorman, G. A.; Harris, M. W.; Moore, J. A. (1980b) Laboratory studies on polybrominated biphenyl-induced immune alterations following low-level chronic and pre-/postnatal exposure. *Int. J. Immunopharmacol.* 2: 69-80.

DRAFT--DO NOT QUOTE OR CITE

- Luster, M. I.; **Germolec**, D. R.; Clark, G.; Wiegand, G.; Rosenthal, G. J. (1988) Selective effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and corticosteroid on in vitro lymphocyte maturation. *J. Immunol.* 140: 928-935.
- Mantovani, A.; Vecchi, A.; Luini, W.; Sironi, M.; Candiani, G. P.; Spreafico, F.; Garattini, S. (1980) Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on macrophage and natural killer cell mediated cytotoxicity in mice. *Biomedicine* 32: 200-204.
- McConnell, E. E.; Moore, J. A.; Haseman, J. K.; Harris, M. W. (1978) The comparative toxicity of chlorinated dibenzo-*p*-dioxins in mice and guinea pigs. *Toxicol. Appl. Pharmacol.* 44: 335-356.
- Mocarelli, P.; Marocchi, A.; Brambilla, P.; Gerthoux, P.; Young, D. S.; Mantel, N. (1986) Clinical laboratory manifestations of exposure to dioxin in children, a six-year study of the effects of an environmental disaster near Seveso, Italy. *J. Am. Med. Assoc.* 256: 2687-2695.
- Morris, D. L.; Jordan, S. D.; Holsapple, M. P. (1991) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on humoral immunity. I. Similarities to *Staphylococcus aureus* Cowan Strain I (SAC) in the in vitro T-dependent antibody response. *Immunopharmacology* 21: 159-170.
- Morris, D. L.; Snyder, N. K.; Gokani, V.; Blair, R. E.; Holsapple, M. P. (1992) Enhanced suppression of humoral immunity in DBA/2 mice following subchronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Toxicol. Appl. Pharmacol.* 112: 128-132.
- Neubert, R.; Jacob-Muller, U.; Stahlmann, R.; Helge, H.; Neubert, D. (1990) Polyhalogenated dibenzo-*p*-dioxins and dibenzofurans and the immune system. 1. Effects on peripheral lymphocyte subpopulations of a non-human primate (*Callithrix jacchus*) after treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). *Arch. Toxicol.* 64: 345-359.
- Neubert, R.; **Jacob-Muller**, U.; Helge, H.; Stahlmann, R.; Neubert, D. (1991) Polyhalogenated dibenzo-*p*-dioxins and dibenzofurans and the immune system. 2. In vitro effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on lymphocytes of venous blood from man and a non-human primate (*Callithrix jacchus*). *Arch. Toxicol.* 65: 213-219.

DRAFT--DO NOT QUOTE OR CITE

- Neubert, R.; Golor, G.; Stahlmann, R.; Helge, H.; Neubert, D. (1992) Polyhalogenated dibenzo-*p*-dioxins and dibenzofurans and the immune system. 4. Effects of multiple-dose treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on peripheral lymphocyte subpopulations of a non-human primate (*Callithrix jacchus*). Arch. Toxicol. 66: 250-259.
- Neumann, C. M.; Steppan, L. B.; Kerkvliet, N. I. (1992) Distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in splenic tissue of C57Bl/6J mice. Drug Metab. Dispos. 20: 467-469.
- Nikolaidis, E.; Brunstrom, B.; Dencker, L.; Veromaa, T. (1990) TCDD inhibits the support of B-cell development by the Bursa of Fabricius. Pharmacol. Toxicol. 67: 22-26.
- Pazdernik, T. L.; Rozman, K. K. (1985) Effect of thyroidectomy and thyroxine on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induced immunotoxicity. Life Sci. 36: 695-703.
- Poland, A.; Glover, E. (1990) Characterization and strain distribution pattern of the murine Ah receptor specified by the Ah^d and Ah^{b-3} alleles. Mol. Pharmacol. 38: 306-312.
- Poland, A.; Knutson, J. C. (1982) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. Annu. Rev. Pharmacol. Toxicol. 22: 517.
- Poland, A.; Glover, E.; Taylor, B. A. (1987) The murine Ah locus: a new allele and mapping to chromosome 12. Mol. Pharmacol. 32: 471-478.
- Puhvel, S. M.; Sakamoto, M. (1988) Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on murine skin. J. Invest. Dermatol. 90: 354-358.
- Rizzardini, M.; Romano, M.; Tursi, F.; Salmona, M.; Vecchi, A.; Sironi, M.; Gizzi, F.; Benfenati, E.; Garattini, S.; Fanelli, R. (1983) Toxicological evaluation of urban waste incinerator emissions. Chemosphere 12: 559-564.
- Rosenthal, G. J.; Lebetkin, E.; Thigpen, J. E.; Wilson, R.; Tucker, A. N.; Luster, M. I. (1989) Characteristics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induced endotoxin hypersensitivity: association with hepatotoxicity. Toxicology 56: 239-251.
- Silkworth, J. B.; Antrim, L. (1985) Relationship between Ah receptor-mediated polychlorinated biphenyl (PCB)-induced humoral immunosuppression and thymic atrophy. J. Pharmacol. Exp. Ther. 235: 606-611.

DRAFT--DO NOT QUOTE OR CITE

- Silkworth, J. B.; Grabstein, E. M. (1982) Polychlorinated biphenyl immunotoxicity: dependence on isomer planarity and the Ah gene complex. *Toxicol. Appl. Pharmacol.* 65: 109-115.
- Silkworth, J. B.; O'Keefe, P. (1992) Immunotoxicity as a probe of TCDD toxicity in a complex environmental mixture. *The Toxicologist* 12: 238 (Abs. 885).
- Silkworth, J. B.; Antrim, L.; Kaminsky, L. S. (1984) Correlations between polychlorinated biphenyl immunotoxicity, the aromatic hydrocarbon locus, and liver microsomal enzyme induction in C57Bl/6 and DBA/2 mice. *Toxicol. Appl. Pharmacol.* 75: 156-165.
- Silkworth, J. B.; Sack, G.; Cutler, D. (1988) Immunotoxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a complex environmental mixture from the Love Canal. *Fundam. Appl. Toxicol.* 12: 303-312.
- Silkworth, J. B.; Cutler, D. S.; O'Keefe, P. W.; Lipinska, T. (1993) Potentiation and antagonism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin effects in a complex mixture. *Toxicol. Appl. Pharmacol.* 119: 236-247.
- Smialowicz, R. J.; Riddle, M. M.; Williams, W. C.; Diliberto, J. J. (1994) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on humoral immunity and lymphocyte subpopulations: differences between mice and rats. *Toxicol. Appl. Pharmacol.* 124.
- Sonawane, B. R.; Smialowicz, R. J.; Luebke, R. W. (1988) Immunotoxicity of 2,3,7,8-TCDD: review, issues, and uncertainties. Appendix E. EPA Review Draft. A cancer risk-specific dose estimate for 2,3,7,8-TCDD.
- Stehr-Green, P. A.; Naylor, P. H.; Hoffman, R. E. (1989) Diminished thymosin alpha-1 levels in persons exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Toxicol. Environ. Health* 28: 285-295.
- Steppan, L. B.; Kerkvliet, N. I. (1991) Influence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the production of inflammatory cytokine mRNA by C57Bl/6 macrophages. *The Toxicologist* 11: 35 (Abs. 45).
- Sutter, T. R.; Guzman, K.; Dold, K. M.; Greenlee, W. F. (1991) Targets for dioxin: genes for plasminogen activator inhibitor-2 and interleukin-1 β . *Science* 254: 415-418.

DRAFT--DO NOT QUOTE OR CITE

- Takagi, Y.; Aburada, S.; Otake, T.; Ikegami, N. (1987) Effect of polychlorinated biphenyls (PCBs) accumulated in the dam's body on mouse filial immunocompetence. Arch. Environ. Contam. Toxicol. 16: 375-381.
- Taylor, M. J.; Clark, G. C.; Atkins, Z. Z.; Lucier, G.; Luster, M. I. (1990). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin increases the release of tumor necrosis factor-alpha (TNF- α) and induces ethoxyresorufin-o-deethylase (EROD) activity in rat Kupffer's cells (KCs). The Toxicologist 10: 276 (Abs. #1101).
- Theobald, H. M.; Moore, R. W.; Katz, L. B.; Peiper, R. O.; Peterson, R. E. (1983) Enhancement of carrageenan and dextran-induced edemas by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related compounds. J. Pharmacol. Exp. Ther. 225: 576-583.
- Thigpen, J. E.; Faith, R. E.; McConnell, E. E.; Moore, J. A. (1975) Increased susceptibility to bacterial infection as a sequela of exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Infect. Immun. 12:1319-1324.
- Thomas, P. T.; Hinsdill, R. D. (1978) Effect of polychlorinated biphenyls on the immune responses of rhesus monkeys and mice. Toxicol. Appl. Pharmacol. 44: 41-51.
- Thomas, P. T.; Hinsdill, R. D. (1979) The effect of perinatal exposure to tetrachlorodibenzo-*p*-dioxin on the immune response of young mice. Drug Chem. Toxicol. 2: 77-98.
- Tognoni, G.; Bonaccorsi, A. (1982) Epidemiological problems with TCDD (a critical view). Drug Metab. Rev. 13: 447-469.
- Tomar, R. S.; Kerkvliet, N. I. (1991) Reduced T helper cell function in mice exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Toxicol. Lett. 57: 55-64.
- Truelove, J.; Grant, D.; Mes, J.; Tryphonas, H.; Tryphonas, L.; Zawidzka, Z. (1982) Polychlorinated biphenyl toxicity in the pregnant cynomolgus monkey: a pilot study. Arch. Environ. Contam. Toxicol. 11: 583-588.
- Tryphonas, H.; Hayward, S.; O'Grady, L.; Loo, J. C. K.; Arnold, D. L.; Bryce, F.; Zawidzka, Z. Z. (1989) Immunotoxicity studies of PCB (Aroclor 1254) in the adult rhesus (*Macaca mulatta*) monkey - preliminary report. Int. J. Immunopharmacol. 11: 199-206.

DRAFT--DO NOT QUOTE OR CITE

- Tryphonas, H.; Luster, M. I.; Schiffman, G.; Dawson, L. L.; Hodgen, M.; Germolec, D.; Hayward, S.; Bryce, F.; Loo, J. C. K.; Mandy, F.; Arnold, D. L. (1991a) Effect of chronic exposure of PCB (Aroclor 1254) on specific and nonspecific immune parameters in the rhesus (*Macaca mulatta*) monkey. *Fundam. Appl. Toxicol.* 16: 773-786.
- Tryphonas, H.; Luster, M. I.; White, K. L., Jr.; Naylor, P. H.; Erdos, M. R.; Burleson, G. R.; Germolec, D.; Hodgen, M.; Hayward, S.; Arnold, D. L. (1991b) Effects of PCB (Aroclor 1254) on non-specific immune parameters in Rhesus (*Macaca mulatta*) monkeys. *Int. J. Immunopharmacol.* 13: 639-648.
- Tucker, A. N.; Vore, S. J.; Luster, M. I. (1986) Suppression of B cell differentiation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Mol. Pharmacol.* 29: 372-377.
- van Logten, M. J.; Gupta, B. N.; McConnell, E. E.; Moore, J. A. (1980) Role of the endocrine system in the action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the thymus. *Toxicology* 15: 135-144.
- Vecchi, A.; Mantovani, A.; Sironi, M.; Luini, M.; Cairo, M.; Garattini, S. (1980) Effect of acute exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on humoral antibody production in mice. *Chem. Biol. Interact.* 30: 337-341.
- Vecchi, A.; Sironi, M.; Canegrati, M. A.; Recchis, M.; Garattini, S. (1983) Immunosuppressive effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in strains of mice with different susceptibility to induction of aryl hydrocarbon hydroxylase. *Toxicol. Appl. Pharmacol.* 68: 434-441.
- Vos, J. G.; Luster, M. I. (1989) Immune alterations. In: Kimbrough, R. D.; Jensen, A. A., eds. *Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products*. Amsterdam: Elsevier Science Publishers B.V. (Biomedical Division); pp. 295-322.
- Vos, J. G.; **Moore**, J. A. (1974) Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Int. Arch. Allergy* 47: 777-794.
- Vos, J. G.; van Driel-Grootenhuys, L. (1972) PCB-induced suppression of the humoral and cell-mediated immunity in guinea pigs. *Int. Arch. Allergy* 47: 777-794.
- Vos, J. G.; Moore, J. A.; Zinkl, J. G. (1973) Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the immune system of laboratory animals. *Environ. Health Perspect.* 5: 149-162.

DRAFT--DO NOT QUOTE OR CITE

- Vos, J. G.; Moore, J. A.; Zinkl, J. G. (1974) Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in C57Bl/6 mice. *Toxicol. Appl. Pharmacol.* 29: 229-241.
- Vos, J. G.; Kreeftenberg, J. G.; Engel, H. W. B.; Minderhoud, A.; Van Noorle Jansen, L. M. (1978) Studies on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced immune suppression and decreased resistance to infection: endotoxin hypersensitivity, serum zinc concentrations and effect of thymosin treatment. *Toxicology* 9: 75-86.
- Webb, K. B.; Evans, R. G.; Knutsen, A. P.; Roodman, S. T.; Roberts, D. W.; Schramm, W. F.; Gibson, B. B.; Andrews, J. S.; Needham, L. L.; Patterson, D. G. (1989) Medical evaluation of subjects with known body levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Toxicol. Environ. Health* 28: 183-193.
- Weissberg, J. B.; Zinkl, J. G. (1973) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin upon hemostasis and hematologic function in the rat. *Environ. Health Perspect.* 5: 119-123.
- White, K. L.; Lysy, H. H.; McCay, J. A.; Anderson, A. C. (1986) Modulation of serum complement levels following exposure to polychlorinated dibenzo-*p*-dioxins. *Toxicol. Appl. Pharmacol.* 84: 209-219.
- Whitlock, J. P. (1990) Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin action. *Annu. Rev. Pharmacol. Toxicol.* 30: 251-277.
- Wood, S. C.; Holsapple, M. P. (1993a) Direct suppression of superantigen-induced IgM secretion in human lymphocytes by 2,3,7,8-TCDD. *Toxicol. Appl. Pharmacol.* 122: 308-313.
- Wood, S. C.; Karras, J. G.; Holsapple, M. P. (1992) Integration of the human lymphocyte into immunotoxicological investigations. *Fundam. Appl. Toxicol.* 18: 450-459.
- Wood, S. C.; Jeong, H. G.; Morris, D. L.; Holsapple, M. P. (1993b) Direct effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in human tonsillar lymphocytes. *Toxicology* 81: 131-143.
- Yang, Y. G.; Lebrec, H.; Burleson, G. R. Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on viral replication and natural killer (NK) activity in rats (submitted).