EPA/600/AP-92/001c
August 1992
Workshop Review Draft

Chapter 3. Acute, Subchronic, and Chronic Toxicity

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

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Please note that this chapter is a preliminary draft and as such represents work in progress. The chapter is intended to be the basis for review and discussion at a peer-review workshop. It will be revised subsequent to the workshop as suggestions and contributions from the scientific community are incorporated.

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LIST OF ABBREVIATIONS

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

LIST OF ABBREVIATIONS (cont.)

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

LIST OF ABBREVIATIONS (cont.)

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

LIST OF ABBREVIATIONS (cont.)

NOEL	No-observed-effect level
OCDD	Octachlorodibenzo-p-dioxin
OCDF	Octachlorodibenzofuran
PAH	Polyaromatic hydrocarbon
PB-Pk	Physiologically based pharmacokinetic
PCB	Polychlorinated biphenyl
OVX	Ovariectomized
PBL	Peripheral blood lymphocytes
PCQ	Quaterphenyl
PeCDD	Pentachlorinated dibenzo-p-dioxin
PeCDF	Pentachlorinated dibenzo-p-dioxin
PEPCK	Phosphopenol pyruvate carboxykinase
PGT	Placental glutathione transferase
PHA	Phytohemagglutinin
PWM	Pokeweed mitogen
ppm	Parts per million
ppq	
ppt	Parts per trillion
RNA	Ribonucleic acid
SAR	Structure-activity relationships
SGOT	Serum glutamic oxaloacetic transaminase

LIST OF ABBREVIATIONS (cont.)

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

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The Office of Health and Environmental Assessment (OHEA) within the Office of Research and Development was responsible for the preparation of this chapter. The chapter was prepared through Syracuse Research Corporation under EPA Contract No. 68-CO-0043, Task 20, with Carol Haynes, Environmental Criteria and Assessment Office in Cincinnati, OH, serving as Project Officer.

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3. ACUTE, SUBCHRONIC AND CHRONIC TOXICITY

3.1. SCOPE AND LIMITATIONS

The acute, subchronic and chronic toxicology of the chlorinated dioxins, dibenzofurans, biphenyl 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 dioxin-like compounds when relevant data are available. The chapter does not have the intention to reference all published material but rather seeks to select 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 will all be dealt with in separate chapters, nor will ecotoxicology be covered in this chapter.

3.2. ACUTE TOXICITY

The range of doses of TCDD which are lethal to animals varies extensively both with 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). More than an 8000-fold difference exists between the dose of TCDD reported to cause 50% 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 vision*) and for the male an LD_{50} value of only 4.2 μg/kg has been calculated (Hochstein et al., 1988).

Among traditional experimental animals, the rat seems to be the second most sensitive species, although there is a >300-fold variability in LD_{50} values between different strains. The Han/Wistar (Kupio) strain of rat has been shown to be particularly resistant to TCDD exposure (Pohjanvirta and Tuomisto, 1987).

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)	*per os*	0.6-2.1	5-34	NR 30	50	McConnell et al., 1978b; Schwetz et al., 1973
Mink/NR (male)	*per os*	4.2	7-17	28	31	Hochstein et al., 1988
Chicken/NR (NR)	*per os*	<25	12-21	NR	NR	Greig et al., 1973
Monkey/rhesus (female)	*per os*	~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)	*per os*	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)	*per os*	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)	*per os*	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)	*per os* 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} (μg/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)	*per os*	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

[a]Of succumbed animals

[b]Mean time to death

[c]Data from five animals

NR = Not reported

Among the five rats per dose group (0, 1500, 2000, 2500 or 3000 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 which addresses the effect of age at the point of exposure to TCDD are scarce, and comparisons are hampered by the absence of 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 vehicle used or the presence of other substances that affect uptake.

The differences in sensitivity towards 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 LD_{50} values of 159 and 3351 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 for the wild-type mice carrying two copies of the "b" gene.

In contrast, the two strains of rats studied by Pohjanvirta et al. (1988) [i.e., Long-Evans and Han/Wistar (Kuopio)] had intraperitoneal LD_{50} values of 10 and >3000 μg TCDD/kg, although no differences as regards the amount or the affinity of available Ah receptor could be found.

Geyer et al. (1990) utilized both their own and other data to determine a correlation between total body fat content and the acute toxicity in various species and strains of laboratory mammals. However, data from the Han/Wistar (Kuopio) rats that are extremely resistant to TCDD-induced lethality (Pohjanvirta and Tuomisto, 1987) were not included. They found a correlation of 0.834 and

suggested that the reasons for this correlation is obvious that an increased total body fat content represents an enhanced capacity to remove TCDD from the systemic circulation. This factor may be important, but it almost certainly doesn't explain all the interspecies differences.

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% 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 most sensitive markers 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, and the cutaneous lesions closely mimic the chloracne and hyperkeratosis observed in humans. The histopathological alterations observed 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 tissue (Peterson et al., 1984) and of the 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. Lethal doses to guinea pigs do not result in liver

TABLE 3-2

Toxic Responses 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	++	0	+[c]	0	0	++		0
skin								
Hypoplasia, atrophy or necrosis	+	+	+	+	+		+	+
thymus	+	+			±		+	
bone marrow	+	+		+	+			
testicle								
Liver lesions	+	±	++	+	++	+	+	±
Porphyria	0	0		+	++		+	0
Edema	+	0		0	+		++	+

[a]Sources: 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; Schwetz et al., 1973; Vos and Beems, 1971; Vos and Koeman, 1970

[b]Responses followed exposure to 2,3,7,8-TCDD or structurally-related chlorinated aromatic hydrocarbons.

[c]Skin 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

damage, which is comparable to the liver lesions described in rabbits and rats, or to 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; however, the ED_{50} 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 the bone marrow hematopoiesis in mice, both *in vivo* and *in vitro*, by directly altering the colony growth efficiently 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). Details of all underlying studies for these observations have been extensively reviewed (U.S. EPA, 1984, 1985; WHO/IPCS, 1989).

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 μg/kg intraperitoneally). a significantly decreased beta-adrenergic responsiveness was observed in the right ventricular papillary muscle of 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 to increases in extracellular calcium were enhanced in these animals. Based on these findings, the authors suggested 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 which have been observed in cases of human intoxications (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 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) and AHH EROD activity has been shown to be induced *in vitro* (see Section 3.5.4).

Wiebel et al. (1991) have identified a cell line (H4IIEC3-derived 5L hepatoma cells) which 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 also lacked measurable amounts of the Ah receptor (Göttlicher et al., 1990). In addition, 3,3',4,4'-TCB also 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 symptom is in general agreement, 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 on 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/CDFs (Plüess et al., 1988). Adult male and female Sprague-Dawley 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 continued for post-exposure observation. 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 post-treatment 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 Sprague-Dawley 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 the subchronic toxicity and the depletion of hepatic vitamin A reduction followed the rank order of the ability of the compounds to bind to the Ah receptor or cause

induction of AHH, for example (Plüess et al., 1988; 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 Sprague-Dawley 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 μg/kg/week.

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 μg/kg bw/day.

In male and female Hartley guinea pigs, a 90-day feeding study of TCDD has been performed by DeCaprio et al. (1986) where extensive pathology, hematology and serum chemistry on surviving animals were performed. The diets contained 0, 2, 10, 76 or 430 ng TCDD/kg. 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). Signs of toxicity such as body weight loss, epidermal changes and anemia occurred 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 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 have utilized initial loading doses, and due to the long half-life of TCDD, steady states may not 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 the dose levels (see Chapter 1), which further complicates any comparisons.

Irrespective 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 study of Kociba et al. (1978, 1979). Groups of 50 male and 50 female Sprague-Dawley 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 μg/kg/day, while increased mortality was not observed in male rats at this dose, or in animals receiving doses of 0.01 or 0.001 μg/kg/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 μg/kg/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 to 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 μg/kg/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 to the end of the study revealed that liver 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

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 µg/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 µg/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 µg/kg/week (males) 0.04, 0.2, 2.0 µg/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

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 μg/kg/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 high-dose groups, respectively. The LOAEL in this study was estimated to be 0.001 μg/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 μg/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., 1979) have 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., ~2-3 ng/kg bw/day for 20 months (Schantz et al., 1979).

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 have been observed at the lowest dose tested, ~2-3 ng/kg body weight.

3.5. SPECIFIC EFFECTS

3.5.1. Wasting syndrome. TCDD at high doses (lethal or near lethal) causes a starvation-like 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, 1989), but no single explanation has been obtained so far. No generalized impairment of intestinal absorption seems to occur. Peterson et al. (1984) has suggested a model for the TCDD-induced wasting syndrome which is based on the assumption that body weight in rats is regulated around an internal standard or a 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 Sprague-Dawley rats. Additionally, TCDD-treated rats maintain and defend their reduced weight level with the same precision as *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 LD_{80} 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 Sprague-Dawley 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 LPL, which controls the serum level of triglycerides. Brewster and Matsumura (1984) found in guinea pigs that the LPL activity was decreased to 20% of the value of *ad libitum* fed controls after

1 day, and this effect persisted throughout the study (10 days). Thus, the authors suggested that TCDD irreversibly reduces adipose LPL activity, thus making the animals less capable to adapt 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 μg/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 also 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 effects observed (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 Fisher 344N rats given a single oral TCDD dose of 100 μg/kg (Max and Silbergeld 1987).

Another biochemical effect noted in TCDD-induced wasting is the ability of TCDD to decrease hepatic vitamin A storage in 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 μg/kg intraperitoneally to male Sprague-Dawley 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 suggested 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 in the median eminence. This suggested 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 starts is preceded by the inhibition of PEPCK, which caused a reduction in gluconeogenesis. This was followed by a progressive increase in plasma tryptophan levels which was suggested to cause a serotonin-mediated reduction of the feed intake. In Sprague-Dawley rats, TCDD in doses of 25 and 125 μg caused a rapid decrease (50%) in PEPCK activity 2 days after dosing, which was 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, increase in the hepatic smooth endoplasmatic reticulum and parenchymal cell necrosis. The histological findings are accompanied by hyperbilirubinemia, hypercholesterolemia, hyperproteinemia, increased SGOT and SGPT activities, further indicating damaged liver function (WHO/IPCS, 1989). These lesions may be severe enough to be a contributing factor in death. The lesions observed after sublethal doses are qualitatively almost identical to those 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 nonresponsive mice (C57BL/6J and DBA/2J, respectively). C57BL/6J mice given a single dose of 3 μg/kg TCDD developed mild to moderate hepatic lipid accumulation but no inflammation or necrosis. Severe fatty change, mild

inflammation and necrosis occurred at 150 μg/kg. DBA/2J mice given 30 μg/kg developed hepatocellular necrosis and inflammation but no fatty change. Lipid accumulation was only slight after 600 μg/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 in 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% of the control level were 1, 14 and 32 μg/kg for the guinea pig, the Sprague-Dawley rat and the Syrian Golden hamster, respectively (Madhukar et al., 1984). A single intraperitoneal dose of 115 μg TCDD/kg bw decreased the EGF binding by 93.1, 97.8 and 46.0% 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 congenic mice of the strain C57BL/6J (Lin et al., 1991a,b). The ED_{50} for the TCDD-induced decrease in the 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 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 copropporphyrin 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 Sweeny, 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 Sweeny, 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 studied 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 to controls. In contrast, the Ah^d mice only showed a 6-fold 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 doses 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 ~0.1 μg 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 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 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 upon 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 "dioxin-like" activities of both environmental samples and of 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 nmol concentrations with a variety of signs indicating alterations in the normal differentiation program (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 the most extensively studied biochemical responses produced by TCDD. The 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 requiring metabolic transformation by the MFO system before they can exert their toxic effect. 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), which are multi-functional enzyme systems involved in conjugating a wide variety of structures and play a key role in biotransformation and detoxification of endogenous and exogenous compounds.

Several investigators have studied the relative potency of various halogenated dioxins, dibensofurans 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 good 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 by it. The measurement 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 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-450lA1 and cytochrome P-450lA2, 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 ß-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 have revealed that enzyme induction, due to TCDD exposure is also 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 >3000 μg, respectively). Differences in the inducibility of EROD, ECOD or ethylmorphine N-demethylase were not found, nor were there any differences as regards the amount of available Ah receptor or the amount of cytochromes 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 which are genetically different at the Ah locus, thus making them responsive or nonresponsive to the induction of hepatic cytochrome P-4501A1 related enzyme activities. Qualitatively and in general, the same responses can be obtained in both strains, but there may be more than one order of magnitude difference as regards the doses required to elicit a response. TCDD is thus 10-fold more potent in inducing hepatic cytochrome P-4501A1 and the related AHH activity in C57BL/6J mice (Ah-responsive) than in DBA/2 mice (Ah-nonresponsive) (Poland and Knutson, 1982; Nebert, 1989).

The guinea pig, although it is the species most sensitive to the toxic effects of TCDD, does not respond to the administration of TCDD with liver toxicity nor 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 as regards increases in cytochromes P-450 (Hook et al., 1975; Liem et al., 1980).

Similarly, hepatic enzyme induction has only been 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 ED_{50} 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 on EROD induction in guinea pigs, rats, C57BL/6 and DBA/2 mice, as well as 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 to 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*) utilizing ^{14}C-labeled caffeine and measuring $^{14}CO_2$ 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 stated that the N-demethylation of caffeine probably was P-4501A1 dependent, studies by Butler et al. (1989) indicate that this reaction is dependent on cytochrome P-4501A2.

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 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 studied in 11 established cell lines and in fetal primary cultures from five animal species and cultured human lymphocytes and 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 have been shown to be inducible for AHH activity by TCDD [e.g., 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)].

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), which is extremely sensitive to AHH induction. The EC_{50} values for AHH- and EROD-induction in the same cell system varied over seven 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).

The feasibility of using *in vitro* EROD induction to determine dioxin-like activities of environmental samples has been demonstrated by several studies (Zacharewski et al., 1989; Hanberg et al., 1991). However, in environmental samples there exists a variety of compounds which bind to the Ah receptor. Some of them might act as antagonists to the binding of CDDs and CDFs and, thus, give an erroneous result. The synthetic compound, 6-methyl-1,3,8-trichlorodibenzofuran, has been shown to inhibit the binding of TCDD to the Ah receptor and to antagonize the induction of both P-4501A1 and P-4501A2 in the rat (Astroff et al., 1988).

3.5.5.1. Appraisal. Based on the 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 towards 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 to 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 steroid-

like actions 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) have 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 lower in adrenal ectomized Sprague-Dawley rats, compared to 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 congenic 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%. Differences in dose-response curves between the different strains could not be observed. These data suggests 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) suggested 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 Sprague-Dawley (S-D) and Gunn rat, the latter being defective in producing some UDPGTs. The homozygous Gunn rat is 3-10 times more resistant to effects of TCDD than is the S-D 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).

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). 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 has suggested 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 Sprague-Dawley rats, when compared to pair-fed and *ad libitum* fed controls. The ED_{50} for this effect was ~15 μg/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 μg/kg was found to cause a 55% decrease in testicular cytochrome $P\text{-}450_{scc}$ activity but also to cause the inhibition of the mobilization of cholesterol to cytochrome $P\text{-}450_{scc}$. The authors concluded that the latter effect probably was responsible for the inhibition of testicular steroidogenesis

(Moore et al., 1991). Despite this, the effects noted occur after exposure to large amounts of TCDD. In contrast, maternal exposure to TCDD has been shown to affect the male reproductive system at much lower 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 TCDD-induced induction of cytochromes P-450 species results in a major increase in the NADPH-dependent metabolism of arachidonic acid (Rifkind et al., 1990). These effects are thus clearly related to the 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 "dioxin-like" 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. TCDD is unique in its ability to reduce the vitamin A content of the liver, both regarding the minute quantities needed to produce this effect and the persistence of the effect. 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 Sprague-Dawley 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% 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, suggestive of 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 μg 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 μg 3 times/week for 3 and 2 weeks, respectively). In the haired phenotype, 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 liver levels of vitamin A. TCDD-induced body weight loss and atrophy of the thymus glands was 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 S-D 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 μg/kg) to female S-D rats reduced vitamin A in the liver, the lung, the intestines and the adrenal glands while increasing its concentration in the serum, the kidneys and the urine. They also found a 150% 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 which is accompanied by an enhanced elimination of the vitamin via the kidney into the urine.

In a comparative study of TCDD toxicity in male S-D rats and Hartley guinea pigs (Håkansson et al. (1989b), the animals were given single intraperitoneal doses of 40 and 0.5 μg/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 concentration 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 also 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, the kidney, the testicles or the serum after a sublethal dose (4 μg/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 apply to these strains of rats.

The interaction of 3,4,3',4'-TCB with vitamin A has been studied by Brouwer and Van der Berg (1983, 1984, 1986), Brouwer et al. (1985, 1986a,b) and Brouwer (1987). The effects of TCB on vitamin A differs in many respects from those of TCDD. TCB is rapidly converted *in vivo* into a polar 5-OH-TCB metabolite, and this metabolite 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 (1985) 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. As 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. On the other hand, a dioxin-like PCB such as 3,4,3',4'-TCB seems to deviate with regard to this mechanism of action.

3.5.8. Lipid Peroxidation. Lipid peroxidation and oxidative stress have been indicated as a factor that affects 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. A characteristic of TCDD toxicity is also the delay required to manifest toxicity (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, and sensitivity to the ulcerative action of DMBA 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 a 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 LD_{50} in this strain of mice is at least 5-fold 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 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 F_1 offspring were as resistant to TCDD toxicity as the Han/Wistar rats (LD_{50}, >3000 μg/kg). Further studies on the F_2 generation indicated that the distribution of resistant and susceptible phenotypes were consistent with inheritance regulated by two (possibly three) autosomal genes displaying complete dominance, independent segregation and an additive co-effect. 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

arise 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 LD_{50} values for guinea pig, rat, mouse, rabbit and hamster, the amounts of 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 LD_{50} values for TCDD vary >100 times between the chick embryo, the C3H/HeN mice and the Sprague-Dawley rat, the ED_{50} doses for AHH induction in these species are comparable (Poland and Glover, 1974). Even in strains of rats with a difference of >300 times in LD_{50}, 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 which reduces the body weight to a maintenance level 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 amply evident that sometimes there are enormous differences in the sensitivity to specific TCDD-induced toxicities. This conclusion is valid for almost all the responses studied. However, qualitatively there seems to be fairly good agreement between the type of responses that can be recorded (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 occurring.

- Our present knowledge, however, 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 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 which 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 altered homeostasis of endogenous compounds caused by the TCDD-induced increased activities of various enzymes.

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	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
C578/6 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
Sprague-Dawley rat	1 ng/kg-day for 2 years in feed	histopathologic alterations	Kociba et al., 1978

[a]0.6 ng/kg = no effect level

- It has repeatedly been reported that the current opinion is that all known effects of TCDD are probably Ah receptor mediated (e.g., Roberts, 1991). Except for the chain of events leading to the induction of certain enzymes, clear evidence for such a conclusion is still lacking. However, the studies in congenic mice in combination with the usually rather strong correlation between enzyme induction and various other TCDD-induced toxic responses makes the assumption rather likely. Further support for the probability of a receptor-mediated process is provided by the very strong structure-activity relationship which has been demonstrated between various CDDs/CDFs and a variety of toxic responses.

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