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 have been reviewed extensively in recent years. This chapter summarizes knowledge on the toxicology of tetrachlorodibenzo-*p*-dioxin (TCDD), but also includes references to other dioxin-like compounds when relevant data are available. Included are selected various data that are considered to be of importance to risk assessment, particularly experimental animal data. Immunotoxicity, reproductive/developmental toxicity, carcinogenicity, toxicity to humans, and epidemiology are all covered in other chapters. Ecotoxicology is not covered in this chapter, but examples from mammalian and avian laboratory species are included.

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). One of the characteristics of TCDD-induced toxicity is delayed manifestation of lethality after acute exposure, with the time to death after exposure being several weeks. Death usually occurs as a consequence of loss of body weight (wasting syndrome) from TCDD-induced inhibition of gluconeogenesis and appetite suppression. Deaths within the first week after exposure—an unusually rapid course for TCDD toxicity—have been observed in guinea pigs (Schwetz et al., 1973), rabbits (Schwetz et al., 1973), and Syrian Golden hamsters (Olson et al., 1980). A more than 8,000-fold difference exists between the dose of TCDD reported to cause 50% lethality (LD_{50}) in male Hartley guinea pigs, the most sensitive species tested (Schwetz et al., 1973), and the LD_{50} dose in male Syrian Golden hamsters (Henck et al., 1981). Another animal with extremely high sensitivity is the mink (*Mustela vision*); for the female, the calculated 28-day LC_{50} value is 0.264 µg TCDD/kg bw/day (Hochstein et al., 1998), which is an order of magnitude less than the 28-day LD_{50} of 4.2 µg TCDD/kg bw/day for male mink (Hochstein et al., 1988).

The rat seems to be the third most sensitive species among experimental animals, although there is a >300-fold variability in LD_{50} values among different strains. The Han/Wistar (H/W) 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 40-day observation period. The DBA/2 male mouse has also been shown to have a high resistance to TCDD toxicity (Chapman and Schiller, 1985).

Data on gender differences in sensitivity to the lethal effects of TCDD are conflicting. The gender differences in the acute toxicity of TCDD are likely due to differences in toxicokinetics, i.e., higher tissue concentrations and longer half-life in females than in males (Li et al., 1995). Acute toxicity data that address the effect of age at the time of exposure to TCDD are scarce, and comparisons are hampered by either the absence or inadequacy of information on the age and body weight of the tested animals. As demonstrated with other chemicals, the acute toxicity of TCDD may vary several-fold depending on the vehicle used or the presence of other substances that affect uptake.

Differences in sensitivity toward TCDD among various strains of mice have been shown 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 3,351 µg/kg for wild-type mice ($Ah^{b/b}$) and congenic mice ($Ah^{d/d}$), respectively. The mean time to death, 22 days, 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. The relative dose needed to bring about various acute responses, however, 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.

The DBA/2 mouse strain requires 10 to 20 times higher doses of 2,3,7,8-TCDD than does the C57BL/6 strain for lethality (Chapman and Schiller, 1985). The reason for this difference between the two strains is the low TCDD-binding affinity to the Ah receptor in the DBA/2 strain (Okey et al., 1994). The difference in ligand-binding affinity, associated with susceptibility to TCDD-induced lethality that segregates with the Ah locus (Chapman and Schiller, 1985), is due to a point mutation (translated as alanine to valine) in the ligand-binding domain of codon 375 (Poland et al., 1994; Ema et al., 1994).

Wasting, hemorrhage, and anemia are the three primary causes for dioxin-induced lethality in rats, and a body weight loss of 25% is considered to be the minimum threshold to assign the presence of wasting syndrome for rats (Viluksela et al., 1997a,b, 1998).

1,2,3,4,5,6,7,8-HeptaCDD (HpCDD)-induced dose-response for wasting and hemorrhage overlap in female Sprague-Dawley rats (Rozman, 1999). Death from wasting and hemorrhage occur within the first few weeks of exposure. Animals that did not exhibit wasting or hemorrhage died from anemia, which did not start before day 126 postexposure (Rozman, 1999). Furthermore, unlike rats dying of wasting syndrome, the ones dying of anemia or hemorrhage had fat depots in the body, suggesting that increased body fat may aid them in surviving beyond the 30-day mark (Rozman,1999), only to succumb later to hemorrhage and anemia.

Long-Evans (L-E) *Turku* AB strain rats are around 1000-fold more sensitive to TCDD-induced acute lethality (LD_{50} about 10 µg/kg) than H/W Kuopio strain rats ($LD_{50} > 9,600$ µg/kg)

(Pohjanvirta et al., 1999). This feature of the H/W rat being highly resistant to acute TCDD toxicity, yet sensitive to enzyme induction, may be due in part to differences in AhR types between rat strains. The H/W strain has point mutations at exon 10 and at the first invariant nucleotide at the 5' end of intron 10 in the AhR gene. These cause alterations in AhR protein structure, leading to loss and alteration of multiple amino acid sequences at the carboxyl terminal region of the transactivation domain (Pohjanvirta et al., 1998). The homozygous AhR [hw/hw] type fails to mediate some endpoints of TCDD toxicity that parallel lethality. At lethal doses, H/W rats show only slight changes in bilirubin and body weight, while L-E rats show a five-fold increase in bilirubin and a 20% to 30% decrease in body weight as early as 6 days postexposure (Unkila et al., 1994a). Furthermore, at lethal doses H/W rats manifest only slight or transient inhibition of daily food intake and body weight gain, whereas in L-E rats progressive decrease in daily feed intake and body weight gain occur within 4 to 7 days postexposure (Unkila et al., 1994a).

Tuomisto et al. (1999) suggested that an uncharacterized gene, other than AhR, determines resistance of H/W Kuopio rats to TCDD-induced acute toxicity.

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 (TBF) may enhance the capacity to remove TCDD from the systemic circulation. This factor may be important, but it almost certainly does not explain all of the interspecies differences. Geyer et al. (1997) have determined that there is a linear relationship in mammals independent of strain and species between the logarithm of the oral 30-day LD_{50} in units of µg/kg bw and the mammal's TBF in percent via the regression equation:

$$\log LD_{50} = 5.30 \times \log TBF - 3.22$$

Data from studies of H/W Kuopio rats, which are extremely resistant to TCDD-induced lethality (Pohjanvirta and Tuomisto, 1987), were not included in this equation.

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 10 or 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).

The female mink (*Mustela vision)* is more sensitive than the male mink to TCDD-induced lethality. Hochstein et al. (1998) fed 2- or 3-year-old adult female mink diets

supplemented with 0, 0.001, 0.01, 0.1, 1, 10, or 100 ppb TCDD for up to 125 days. Feed consumption was significantly depressed in the 10 and 100 ppb groups beginning in weeks 4 and 3, respectively. When adjusted for body weight (g food intake/100 g bw/day), the feed intake in TCDD-exposed groups was not significantly different from the control, except in the 10 ppb-dosed group during week 5. Significant body weight loss associated with classic symptoms of wasting syndrome resulting in mortality was observed in the 1, 10, and 100 ppb-dosed groups, respectively, from the third, second, and first week of exposure. Mortality reached 12.5%, 62.5% and 100% by day 28 in the 1, 10, and 100 ppb-dosed groups, respectively. By day 125, mortality increased to 62.5% and 100% in the 1 and 10 ppb groups. Based on the average feed intake of 5.5 g/100 g bw/day for the control mink, the dietary LC_{50} values of 4.8 and 0.85 ppb approximate 0.264 and 0.047 µg TCDD/kg bw/day, respectively, for 28 and 125 days of exposure.

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 database is derived from high-dose effects. The liver is the organ primarily affected in rodents and rabbits, while atrophy of the thymus and lymphatic tissues seems to be the most sensitive marker of toxicity in guinea pigs (WHO/IPCS, 1989; U.S. EPA, 1984, 1985). It is not possible to specify a single organ whose dysfunction accounts for lethality. Dermal effects are prominent signs of toxicity in nonhuman 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 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.

Loss of body weight, or wasting syndrome, is a characteristic sign observed in most animals exposed to 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., 1978a; 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 ED_{50} for increased hepatic weight is only ~15 µg/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 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 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).

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 lethal dose of TCDD (10 µg/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, enhanced responsiveness to low-frequency stimulation and increases in extracellular calcium were observed in these animals. Based on these findings, the authors suggest that the heart may be a major target for TCDD lethality at acutely toxic doses.

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., 1978b). These symptoms are similar to those observed in cases of human intoxication, such as from occupational exposure, the Seveso incident, and the Yusho and Yu-Cheng toxic oil intoxications, the latter involving exposure to PCBs and CDFs (see Chapter 7).

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 were few effects documented on these general cellular parameters in early studies.

Other in vitro studies, using more specific endpoints of toxicity, have clearly indicated effects of TCDD at comparatively low concentrations. For example, 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).

TCDD was found to inhibit high-density growth arrest in human squamous carcinoma cells in culture (Hebert et al., 1990a). Wiebel et al. (1991) identified a cell line (H4IIEC3-derived 5L hepatoma cells) that responds with decreased proliferation at low TCDD concentrations. In this cell line, half-maximum inhibition of proliferation occurs at a concentration of 0.1-0.3 nM. The onset of the effect is fairly rapid, manifesting itself as early as 4-8 hours after treatment. Further studies 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, although at higher concentrations.

3.2.3. Appraisal

Numerous studies of acute toxicity in various mammalian species have demonstrated dramatic species- and strain-specific differences in sensitivity. However, most species and strains respond at some level with a spectrum of symptoms that is generally the same, although species differences do 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, including various strains of rats, have demonstrated a wide range of sensitivities regardless of rather comparable levels of the Ah receptor. This in no way obviates the necessary, but not sufficient, role of AhR.

3.3. SUBCHRONIC TOXICITY

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., 1988a,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 overtly from the controls except for a slight increase in the mean liver-to-body weight ratio.

A 13-week dietary study of 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 subchronic toxicity and the depletion of hepatic vitamin A followed the rank order of the ability of the compounds to bind to the Ah receptor and to cause induction of AHH (Plüess et al., 1988a,b; Håkansson et al., 1990). Direct comparisons of the effects are hampered, however, by differences in the toxicokinetic behavior of the compounds. Slightly different relationships with regard to toxicity were observed 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). Both of these studies support the assumption that most signs and symptoms obtained may be mediated through the Ah receptor.

In another study primarily aimed at investigating TCDD-induced porphyria (Goldstein et al., 1982), 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. The animals were killed and studied 1 week after the last treatment. Additional groups of rats received doses of 0 or 1.0 µg/kg/week for 16 weeks and were allowed to recover for 6 months. The high-dose level was lethal to all animals within 12 weeks, while the only other gross sign of toxicity was a decrease in body weight gain in the group receiving 1.0 µg/kg/week. After 16 weeks of exposure to TCDD, liver porphyrins were elevated ~1,000-fold in 7 of 8 animals receiving 1.0 µg/kg/week. Only 1 of 8 animals in the 0.1 µg/kg/week group had elevated porphyrin levels. The no-effect dose for porphyria was 0.01 µg/kg/week. After a 6-month recovery period, the porphyrin level in animals exposed to 1.0 µg/kg/week was still 100-fold higher than the values in the control group. A similar pattern was observed for urinary excretion of uroporphyrin. A 6-month recovery period was not sufficient for complete reversal of TCDD-induced porphyria.

Two studies were conducted (Harris et al., 1973; Vos et al., 1973) in which 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/kg/week, which corresponds to ~0.1 µg/kg bw/day. In a subchronic exposure study, van Birgelen et al. (1996a) observed a synergistic effect of PCBs and TCDD on

hepatic porphyrin in rats at levels comparable with those found in human milk and fat samples. Coadministration of TCDD with 2,2',4,4',5,5'-PCB (PCB 153) resulted in elevated hepatic porphyrin levels not observed in TCDD cotreated with 3,3',4,4',5-PCB (PCB126) or 2,3,3',4,4',5-PCB (PCB156) groups. In this experiment, LOAELs for hepatic porphyrin accumulation for TCDD, PCB126, and PCB 156 were found to be 0.047, 3.18, and 365 µg/kg/d, respectively. van Birgelen et al. (1996b) have further extended the observation on hepatic porphyrin activity in female B6C3F1 mice after subchronic exposure to individual PCDD, PCDF, and PCB congeners. A dose-response relationship with potencies, relative to TCDD, for increased hepatic porphyrin accumulation was observed for all of the individual congeners studied. The relative potencies of PCDDs and PCDFs tested, based on hepatic porphyrin and enzymatic activities associated with hepatic CYP1A1 and CYP1A2, were found to be in a comparable range.

A 90-day TCDD feeding study of male and female Hartley guinea pigs was performed by DeCaprio et al. (1986), in which 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, produced no dose-related alterations. Based on this study, a no-observed-adverse-effect level (NOAEL) 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 did occur, however, in monkeys that 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 no-observable-adverse-effect levels (NOAELs) for rats, mice, and guinea pigs are estimated to be 1 ng, 100 ng, and 0.6 ng TCDD/kg bw/day, respectively. These studies cannot be directly compared with each other, however, and these subchronic NOAELs cannot be used for extrapolating human risk. 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 toward the 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 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 with those of the controls.

Increased urinary coproporphyrin and uroporphyrin were noted in female rats, 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 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 observed 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 (=1 ng/kg/day).

In the National Toxicology Program (NTP, 1982) gavage study of B6C3F1 male and female mice, no adverse effects were seen at the lowest dose tested (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) revealed signs and symptoms similar to those recorded

in more short-term studies. Adverse effects were noted down to the lowest dose tested (~2-3 ng/kg bw/day for 20 months) (Schantz et al., 1979).

3.4.1. Appraisal

From 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 (NOAEL) of 540 ppt. For the male Swiss mouse, dermatitis and amyloidosis in 5 of 44 animals were noted at the lowest dose tested (the LOAEL was 1 ng/kg bw/day). NOAELs of 1.4 and 6 ng/kg/day were obtained for male and female B6C3F1 mice, respectively. The reported studies on rhesus monkeys are problematic for use in such a determination, because adverse effects were observed at the lowest dose tested, ~2-3 ng/kg bw/day.

3.5. SPECIFIC EFFECTS

3.5.1. Wasting Syndrome

TCDD at high doses (lethal or near lethal) causes a starvation-like effect, 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 thus far. No generalized impairment of intestinal absorption seems to occur.

Peterson et al. (1984) conducted behavior experiments and suggested a model for the TCDD-induced wasting syndrome that is based on the hypothesis, advocated by Keesey and Powley (1975, 1986), that body weight in rats is regulated to an internal standard or hypothalamically programmed set-point. According to this hypothesis, the 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 not 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).

Reduced appetite as a result of inhibition of tryptophan-2,3,-dioxygenase causes gradual development of eventual lethal hypoglycemia in TCDD-induced wasting syndrome in rats (Weber et al., 1994). No reduced appetite associated with gradual body weight loss and no tryptophan effects are observed in TCDD-exposed mice, although appetite and body weight loss are observed in mice at the terminal stage of wasting syndrome. 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_{50} 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).

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.

Reduced gluconeogenesis due to inhibition of phosphoenol pyruvate carboxykinase (PEPCK) by TCDD has been suggested as one of the primary contributing factors to a gradual development of an eventual lethal hypoglycemia in wasting syndrome in rats (Stall et al., 1993) and mice (Weber et al., 1995).

TCDD-induced wasting is associated with reduction of adipose tissue mass, hypertriglyceridemia, redistribution of fatty acids (Gasiewicz and Neal, 1979; Chapman and Schiller, 1985; Brewster and Matsumura, 1988), and diabetic-like symptoms (Brewster and Matsumura, 1988). Carbohydrate and lipid metabolism are severely impaired in the liver and adipose tissue by TCDD. Glucose transport systems play vital roles in controlling the rate of energy utilization in adipose tissues. TCDD also affects lipoprotein lipase (LPL) activity. The rate of fat storage is determined by LPL, which controls the serum level of triglycerides. Brewster and Matsumura (1984) found that LPL activity was decreased in guinea pigs to 20% of the value of ad libitum-fed controls after 1 day, and that this effect persisted throughout the study (10 days). The authors suggest that TCDD irreversibly reduces adipose LPL activity, thus making the animals less capable of adapting to nutritional changes and needs. In the pancreas, LPL regulates the production and release of insulin, and in the liver it controls glucose metabolism and fatty acid synthesis. From their observations on the significant reduction of glucose-transporting activity in adipose tissue and pancreas in guinea pigs by TCDD at a very low dose (single IP injection of 0.03 µg/kg), Enan et al. (1992a,b) concluded that the reduction in glucose transporters is one of the major causes of TCDD-induced wasting syndrome in this species. Downregulation of the cellular glucose uptake in NIH 3T3 L1 preadipocyte cell line in culture by TCDD has also been observed (Olsen et al., 1994). Pretreatment of these cells with

4,7,-phenanthroline, an Ah receptor blocker, prevents the effect of TCDD on glucose uptake, suggesting that TCDD-induced downregulation of functional glucose transporter proteins is mediated through the Ah receptor.

The insulin-recruitable form of glucose transporter Type 4 (GLUT4) provides energy to the cell by supplying glucose to the muscle and tissue tissues. Impairment of GLUT4 in adipose tissue, liver, and pancreas (Enan et al., 1992a,b), and reduction of PEPCK in liver (Viluksela et al., 1995), could play important roles in the pathogenesis of TCDD-induced diabetes.

In a series of studies on Wistar rats, Lakshman et al. (1988, 1989, 1991) demonstrated that single intraperitoneal injections of TCDD (from 1 µg/kg) caused a dose-dependent inhibition of fatty acid synthesis in the liver and adipose tissue. Adipose tissue was found to be more sensitive than the liver. 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 of isolated heart mitochondria have indicated that a TCDD concentration of 1.5 nmol/mg in 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, has been observed in male Fischer 344N rats given a single oral TCDD dose of 100 µg/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., 1989a, 1991). Vitamin A is necessary for growth; vitamin A deficiency will result in depressed body weight gain and 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).

The hypothesis that decreased feed intake could be a result of a direct TCDD effect on the brain was initially indicated by Pohjanvirta et al. (1989), although contradictory information has been provided by other studies (Stall and Rozman, 1990). The intraperitoneal administration of TCDD at 50 µg/kg to male SD rats (~LD_{50} level) caused a significant decrease in the serum concentration of prolactin, detectable after 4 hours, compared with pair-fed vehicle controls and noninjected controls (Jones et al., 1987). 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 and the turnover rate were significantly elevated.] This suggests a hypothalamic site of TCDD action in their experiments (Russell et al., 1988), a finding supported by additional data on changes to central thermoregulation by dioxin in golden hamsters (Gordon et al., 1996) and rats (Gordon and Miller, 1998).

Changes in intermediary metabolism have been demonstrated in TCDD-treated experimental animals. Conflicting data on how TCDD affects serum glucose and hepatic glycogen levels have been reported earlier (WHO/IPCS, 1989). Several studies have suggested that the ultimate cause of death in some mammalian species may be a progressive hypoglycemia (Ebner et al., 1988; Gorski and Rozman, 1987; Gorski et al., 1990). Serum glucose levels in the guinea pig, however, were not affected by treatment of the animals with TCDD (Gasiewicz and Neal, 1979). Slight reductions in serum glucose levels were noted in both L-E and H/W 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 µg caused a rapid decrease (50%) in PEPCK activity 2 days after dosing, followed by a dose-dependent decrease in glucose-6-phosphatase activity 4 to 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 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 the extent to which this syndrome is elicited through the interaction of TCDD with the Ah receptor, although 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 to induce the wasting syndrome in both rats and guinea pigs (Safe, 1990).

3.5.2. Hepatotoxicity

Even at sublethal doses, TCDD induces hyperplasia and hypertrophy of parenchymal cells and, thus, hepatomegaly in all species investigated. 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 in the rat and rabbit. The morphological changes in the liver are accompanied by impaired liver function 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).

Hepatotoxic reaction in various strains of rats given lethal doses of TCDD is characterized by degenerative and necrotic changes including 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 endoplasmic reticulum, and parenchymal cell necrosis. The histological findings are accompanied by hyperbilirubinemia, hypercholesterolemia, hyperproteinemia, and increased serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) activities, which further indicate 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 observed after lethal doses.

Early studies in mice found similar effects. More 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). The 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. The 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. This is consistent with the findings of Birnbaum et al. (1990) on the differential toxicity of TCDD in C57BL/6J mice congenic at the Ah locus. In this study, wild type mice were 8- to 24-fold more sensitive than congenic mice deficient at the Ah locus for a spectrum of effects, including increased liver weight, hepatocellular cytomegaly, fatty change, bile duct hyperplasia, and serum liver enzyme changes.

The guinea pig shows less severe morphological alteration in the liver than other species, although ultrastructural changes of the liver are found. 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 membrane function have been studied (U.S. EPA, 1984, 1985; WHO/IPCS, 1989). Adenosine triphosphatase (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 SD 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 the EGF receptor 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 maximum binding capacity of the EGF receptor was 10 times higher in the Ah-nonresponsive mice than the Ah-responsive animals. This study supports the hypothesis that the effects of TCDD on EGF receptor ligand binding are 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 reduced ouabain excretion.

TCDD administration stimulates the accumulation of porphyrins in the liver and an increase in urinary porphyrin excretion (Goldstein et al., 1973, 1976, 1982). 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 has not been elucidated. Cantoni et al. (1981) exposed rats orally to 0.01, 0.1, and 1 µg TCDD/kg bw/week for 45 weeks. 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.

Induction of aminolevulinic acid (ALA)-synthetase, the initial and rate-limiting enzyme involved in heme synthesis, does not seem to be a necessary event in TCDD-induced porphyria. Despite porphyria being evident, mice exposed to 25 µg TCDD/kg bw/week for 11 weeks were not found to have any increased ALA activity (Jones and Sweeney, 1980). 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). Of interest, too, are the results reported in van Birgelen et al. (1996a), where the porphyrinogenic effects of TCDD were correlated with CYPIA2 induction, and demonstrated a strong synergistic relationship with coadministered PCBs.

A comparative study of TCDD-induced porphyria has not been conducted in responsive and nonresponsive mice. In a study on Ah-responsive (Ah[b]) and Ah-nonresponsive (Ah[d]) C57BL/6J female mice, however, 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 than the 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(lA2) 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 common responses to high exposures to TCDD in humans. However, this type of toxicity is expressed only in a limited number of animal species (e.g., rabbits, monkeys, cows, and hairless mice).

In a 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 the rabbit ear bioassay (Knutson and Poland, 1982; Puhvel et al., 1982). Following repeated applications of ~0.1 µg TCDD over several weeks, however, 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 2,3,7,8-TCDD, 2,3,4,7,8-PeCDF, and 1,2,3,4,7,8-HxCDF (Hebert et al., 1990b). 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 doserelated (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 dioxin-like 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. Indeed, the minimum concentration for increases in cell proliferation was 0.1 nM in the most sensitive cell line (SCC-15G). This effect is not due to modulation of the transforming growth factor-β binding (Hebert et al., 1990b,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 mixed-function oxidase (MFO) system is the most thoroughly investigated, and AHH and EROD (as markers for CYPlA1 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 also affects a variety of other enzymes (e.g., uridine diphosphate-glucoronosyltransferase [UDPGT] and glutathione-s-transferase [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 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. 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 a clear correlation between the toxicity and induction potency of a series of CDDs, CDFs, and coplanar PCBs (Poland and Glover, 1973; Safe, 1990). This is discussed in Chapter 2 of this report.

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 CYPlA1) are considered to be very sensitive markers of 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 NOAEL 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). For more detailed dose-response information, see Chapter 8 of this report. 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). TCDD has been shown to increase both the contents of cytochrome P-450lA1 and cytochrome P-450lA2 in the liver, as well as other microsomal enzyme activities involved in the oxidative transformation and conjugation of xenobiotics (e.g., aniline hydroxylase, AHH, biphenyl hydroxylase, 7-ethoxycoumarin-O-deethylase [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, δ-ALA synthetase DT-diaphorase, transglutaminase, ornithine decarboxylase, transaminases (L-alanine aminotransferase [ALT] and L-aspartate aminotransferase [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 of different species have also revealed that enzyme induction due to TCDD exposure varies with both species and strain. Pohjanvirta et al. (1988) studied enzyme induction in the L-E and H/W (Kuopio) rat strains (LD_{50} ~10 and >3,000 µg/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-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 with regard to the doses required to elicit a response. TCDD is thus 10-fold more potent in inducing hepatic cytochrome P-450lA1 and the related AHH activity in C57BL/6J mice (Ah-responsive) than in DBA/2 mice (Ah-nonresponsive) (Poland and

Knutson, 1982; Nebert, 1989) and C57BL/6L mice congenic at the Ah locus (Birnbaum et al., 1990).

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 extensive enzyme induction. Indeed, even at lethal doses, the induction of MFO, as measured by AHH activity, is only very slight (Beatty and Neal, 1977; Håkansson et al., 1994). The data on enzyme induction in rabbits are rather limited and 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 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 (extremely low doses, compared with 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., 1994). This might be due to higher half-life of TCDD in guinea pigs than rats or mice.

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 NOAEL of 1 ng/kg and a LOEL of 3 ng/kg (Kruger et al., 1990). Studies by Butler et al. (1989) and others indicate that this reaction is dependent on cytochrome P-450lA2.

In the chick embryo, both AHH and δ-ALA 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 5 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 have been shown to be inducible for AHH activity by TCDD including lymphocytes (Atlas et al., 1976), squamous cell carcinoma lines (Hudson et al., 1983; Hebert et al., 1990a), 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) 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). Additional details on these and other enzyme induction dose-response characteristics and modeling are included in Chapter 8 of this report.

3.5.4.1. Appraisal

Based on data from Kitchin and Woods (1979), Abraham et al. (1988), Kruger et al. (1990), and Neubert (1991), a NOAEL value of 1 ng/kg bw can be calculated for enzyme induction for both rats and marmoset monkeys. 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 the wide range of sensitivities for enzyme induction both in vivo and in vitro, although the two groups of effects are not necessarily parallel. 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.

3.5.5. Endocrine Effects

In many respects, TCDD toxicity mimics endocrine imbalance. 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 steroid-like activities of TCDD. This has prompted studies on the interaction of TCDD with steroid hormones and their receptors.

Evidence has been provided suggesting that chronic or subchronic exposures to TCDD impair thyroid functions. Dose-dependent reductions of plasma thyroid hormone levels have been observed in TCDD- and PCB-exposed animals (van der Kolk et al., 1992; van Birgelen et al., 1995a,b).

In a subchronic 13-week TCDD feeding study with female Sprague-Dawley (S-D) rats, a decrease in thyroid hormone (T_4) levels occurred, associated with elevation of microsomal UDPGT activity when thyroxine was used as substrate for thyroxine glucuronosyltransferase (T_4UGT) (van Birgelen et al., 1995b). In addition, involvement of CYP1A1 and UGT1A1 by TCDD indicates that the TCDD-induced thyroid functional abnormalities are mediated though the AhR (van Birgelen et al., 1995b). 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156) has also been shown to reduce plasma total T_4 levels, and induces UDPGT by using thyroxine as substrate for T_4UGT (van Birgelen, 1995a). Similar results have also been observed in a 30-week chronic study with female S-D rats suggesting that TCDD-induced thyroid hormone function is caused by chronic perturbation of the liver-pituitary-thyroid axis (Sewell et al., 1995).

van Birgelen et al. (1995a,b) demonstrated the effects of TCDD and coplanar PCB126 (3,3,4,4',5-PCB) on thyroid hormone metabolism in female SD-rats. Oral exposure to 0.2, 0.4, 0.7, 5, and 20 µg/kg diet of TCDD and 7 to 180 µg/kg diet of PCB 126 significantly decreased the plasma total thyroxine (TT_4) levels. An intake of 0.047 µg/kg/day was estimated to be the LOAEL for decrease in plasma thyroid hormone levels.

A dose-dependent decrease in serum T_4 levels has also been observed in male and female SD rats as a result of high-dose subchronic exposures to 1,2,3,7,8-pentaCDD (PeCDD) or 1,2,3,4,7,8-hexaCDD (HxCDD) and low-dose subchronic exposures to TCDD/kg. Serum T_4 levels in PeCDD or HxCDD-exposed males returned to close to normal levels by the end of the off-dose period (Viluksela et al., 1998).

van Birgelen et al. (1995b), in a 13-week TCDD feeding study using 7-week-old female S-D rats, found that the LOAEL for decrease in plasma T_4 was 47 ng/kg bw/day. Dose-response relationships for CYP1A1 and CYP1A2 were determined by nonlinear curve fitting. The critical values for the 95% confidence limits for CYP1A1 and CYP1A2 inductions ranged from 0.7 and 4 ngTCDD/kg bw/day.

Janz and Bellward (1997) reported that a single intraperitoneal dose of 20 µg/kg bw of TCDD to adult great blue heron, Ardea heidias, increased plasma T_4 levels (control: 39 ± 4 ng/mL; exposed: 55 ± 5 ng/mL; $p<0.05$), but no effect occurred on plasma total T_3 levels or on the plasma T_3 to T_4 ratio.

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, it has been suggested that TCDD increases glucocorticoid activity through indirect effects on glucocorticoid receptors. Poland et al. (1976) demonstrated that cortisol and synthetic glucocorticoids do 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 than in control rats (Sunahara et al., 1989). The data further indicate that the binding capacity of hepatic glucocorticoid receptor was altered, but not the apparent equilibrium dissociation constant (K_d). 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%.

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

Early studies reported contradictory data on changes in steroid levels. 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. For example, 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). The results of TCDD exposure in various species and strains are complex. The ability of the strain to counteract TCDD-induced modulation of the estrogen receptor depends on its ability to synthesize and excrete estrogens. Interactions of TCDD and related compounds with estrogen have 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 with the results obtained from long-term bioassays that demonstrated liver tumors only in female rats (Kociba et al., 1978; NTP, 1982).

Studies on congenic strains of Ah-responsive and Ah-nonresponsive C57BL/6J female mice found a statistically significant difference in the responsiveness of the hepatic estrogen receptor. This indicates that the Ah receptor regulates the effects of TCDD on 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; 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). It has also been shown, however, that a single oral dose of 25 µg TCDD/kg bw decreases the plasma corticosterone in

SD rats 14 and 21 days postexposure. It is important 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-ß-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 3α-, 6α-, and 16ß-hydroxytestosterone and an increase of 7α-hydroxytestosterone has also been observed in young male Wistar rats (Keys et al., 1985). Moore et al. (1985) 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 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 100 µg/kg oral dose of TCDD was found to cause a 55 percent decrease in testicular cytochrome P-450$_{scc}$ activity and to inhibit the mobilization of cholesterol to cytochrome P-450$_{scc}$. 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; the lowest dose tested was 64 ng/kg (Mably et al., 1991, 1992a,b,c). This is discussed in 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 clearly related to receptor-mediated enzyme induction.

3.5.6. Vitamin A Storage

Decreased hepatic vitamin A storage has been reported in animals exposed to various chlorinated aromatic compounds. Because only minute quantities are needed to produce ill effects, and because of its persistence in nature, TCDD is unique in its capacity 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% 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. No correlation between the UDPGT activity and the hepatic vitamin A reduction was seen, however, 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).

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

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, the effects of vitamin A depletion by itself were not seen by cutaneous histology, nor were any changes observed in cutaneous morphology attributable to TCDD. 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, in which enzyme-altered hepatic foci were induced in the livers of female SD rats, Flodström et al. (1991) found that vitamin A deficiency by itself enhanced foci development. The effects of TCDD treatment were also markedly enhanced, including TCDD-induced 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 SD rats reduced vitamin A in the liver, lungs, intestines, and adrenal glands, while increasing its concentration in serum, kidneys, and 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, 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., 1989a), 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}). Similar

reductions in hepatic vitamin A were observed for both species, 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, 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 lung correlated reasonably well with other toxic symptoms observed in the animals. On the other hand, studies of two strains of rats, L-E and H/W (the H/W 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 of 4 µg/kg (Pohjanvirta et al., 1990). These findings show that the correlation 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, 1986), 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 transthyretin (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 exposure to this PCB. 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., 1993). Due to the very slow metabolism of TCDD (or other 2,3,7,8-substituted CDDs/CDFs), however, this mechanism probably plays a very minor role in toxicity.

Taken together, these data indicate that TCDD interferes with the metabolism and storage mechanisms for vitamin A (Kelley et al., 1998). Because supplementation of dietary vitamin A seems 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.

3.5.7. 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; Pohjanvirta 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. No studies relating these observations to the Ah receptor have been performed. When considering the available data on TCDD and lipid peroxidation, it is not possible to define a relationship between lipid peroxidation and TCDD-induced lethality. However, oxidative stress is observed only at high doses of TCDD following acute exposure. Acute TCDD exposure at high doses has been shown to produce reactive oxygen species (Alsharif et al., 1994 a,b), lipid peroxidation (Alsharif et al., 1994b), and decreased membrane fluidity (Alsharif et al., 1990) in the mouse and rat.

Oxidative stress has been proposed as one of the reasons for increased susceptibility of female mice to TCDD-induced toxicity. In female C57BL/6J mice, intraperitoneal exposure to 5 µg/kg of TCDD for 3 consecutive days results in a long-term increase in hepatic oxidized glutathione and 8-hydroxydeoxyguanosine levels. Levels of 8-hydroxydeoxyguanosine, a product of DNA base oxidation and subsequent excision repair, remain elevated about 20-fold 8 weeks after treatment. This suggests a sustained TCDD-induced oxidative stress resulting in potentially promutagenic DNA base damage (Shertzer et al., 1998). Induction of CYP1A1 by TCDD has also been suggested to cause an increased excretion rate of 8-oxoguanine, a biomarker of oxidative DNA damage (Park et al., 1996).

Oxidative brain tissue damage may play a role in TCDD-induced central nervous system abnormalities. Hassoun et al. (1998) reported that subchronic oral exposure of B6C3F1 mice to TCDD for 13 weeks can result in a dose-dependent increase in superoxide anions (indicated by reduction in cytochrome c), lipid production, and DNA single-strand breaks in brain tissues. The authors posited involvement of the cytochrome P-450 system in TCDD-induced oxidative stress. Slezak et al. (1999), using CYP1A2 knockout (CYP1A2$^{-/-}$) mice, demonstrated that TCDD-induced oxidative stress (indicated by production of thiobarbituric acid-reactive substances as a measure of lipid peroxidation, production of reactive oxygen species via in vitro reduction of CYC, and changes in glutathione) is not mediated through the cytochrome P-450 type 1A2 isozyme (CYP1A2). Hassoun et al. (1997) also posited that TCDD-induced fetal death and fetal and placental weight reductions in C57BL/6J mice may be caused by oxidative damage induced by TCDD. Ellagic acid at 6 mg/kg/day on days 10, 11, and 12 of gestation and 3 mg/kg on day 13 protected against TCDD administration on day 12 at 30 µg/kg bw. Vitamin E succinate administered at 100 mg/kg/day through gestation days 10, 11, and 12 and at 40 mg/kg on day 13, instead of ellagic acid, was a less effective protective agent.

Iron administered before TCDD administration (75 µg/kg bw) to AhR-responsive AhRb-1 C57BL/6J mice potentiated hepatic porphyria, hepatocellular damage, and plasma hepatic enzyme markers (Smith et al., 1998). The mechanism was oxidative because hydroxylated and peroxylated derivatives of the uroporphyrins formed from uroporphyrinogen,

and μ-glutathione transferase were also induced. Iron overcame the weak porphyria and toxicity responses of TCDD in AhRb-2 BALB/c and AhRd SWR mice, but not in DBA/2 mice, which remained TCDD resistant. Thus, metabolic factors may play a part in the responses of some mice strains to TCDD through an oxidative process that disturbs iron regulatory protein capacity.

Increased accumulation of lipofuscin pigments, which are by-products of lipid peroxidation, in heart muscles of TCDD-exposed rats (Albro et al., 1978) and iron deficiency in animals resulting in in vitro inhibition of lipid peroxidation and reduced TCDD-induced hepatotoxicity (Sweeney et al., 1979) suggested that oxidative stress may play a role in TCDD-induced acute toxicity. Subsequently, Stohs et al. (1983) demonstrated that lipid peroxidation is increased in isolated liver microsomes from TCDD-exposed rats. Further observations suggest a possible role of reactive oxygen species in TCDD acute toxicity. Alsharif et al. (1994a,b) observed that a maximum increase in superoxide anion production occurs on day 1 of posttreatment in female SD rats treated with 50 and 125 µg/kg bw of TCDD, and that TCDD-induced oxidative stress is mediated through the Ah receptor in mice. TCDD-induced superoxide anion production by peritoneal lavage primary macrophages and its mediation through the Ah receptor suggests involvement of reactive oxygen species in a broad spectrum of TCDD-induced toxicity. Bagchi et al. (1993) found that products from altered lipid peroxidation and increased oxidative stress result in elevated serum and urinary levels of certain lipid metabolic products, such as malondialdehyde, formaldehyde, acetaldehyde, and acetone, following a single oral exposure to 50 µg/kg bw of TCDD in female SD rats. Vos et al. (1978) suggested that endotoxin shock may be the cause of TCDD-induced lethality, and that the tumor necrosis factor-α (TNF-α) may be a contributing factor, even though it has not been detected in the serum of TCDD-treated mice without exposure to endotoxin. Alsharif et al. (1994c) demonstrated that anti-TNF-α antibody can decrease phagocytic cell activity following TCDD treatment. This suggests that TNF-α release, a possible activator of TCDD-induced oxidative stress, may have some role in TCDD-induced activation of phagocytic cells.

3.5.8. Neurotoxicity

Exposure to dioxin-like coplanar PCBs may result in neurotoxicity. Eriksson et al. (1991) suggested that dioxin-like coplanar PCBs have neurologic activities that affect the cholinergic receptors in the hippocampus. Seegal (1996) provided evidence that perinatal exposure to coplanar 3,3',4,4'-PCB (PCB 77) results in significant elevation of dopamine in the frontal cortex. Dopamine is an important neurotransmitter, dependent on tyrosine, that is associated with initiation and control of motor behavior, learning, and memory functions. The neurons and astroglia of rat hippocampal neural cells are responsive to relatively low levels of TCDD through mechanisms that are probably not associated with altered gene transcription and

that may involve other cellular targets (Hanneman et al., 1996). TCDD induces phosphorylation and other responses within minutes of treatment, probably through a nonnuclear role of the Ah receptor.

Eriksson and Fredriksson (1998) demonstrated that a single oral exposure of NMRI mice to either 0.51 or 51 mg/kg bw of 3,3',4,4',5,5'-hexachlorobiphenyl (PCB 169) on postnatal day 10 can result in derangement of spontaneous motor behavior. In addition, permanent impairment of learning and working memory was revealed when these animals reached adulthood. This study suggests that exposure to PCB 169 during the neonatal period, at a time when there is incomplete development of the infant's blood-brain barrier and during rapid brain development, can result in vulnerability of the brain to neurologic effects, which in many cases can only be manifested during adulthood. In utero and lactational exposure to TCDD (100 ng/kg/d) or coplanar PCBs resulted in reduction of errors on a radial arm maze working memory task in grown up S-D rats exposed through their mothers (Seo et al., 1999). The effect was more pronounced in males than females. There was no difference in performance of the Morris water maze task or the spatial discrimination-reversal learning task for exposed males and females or unexposed rats. Both adult male and female S-D rats exposed maternally to TCDD showed a deficit in learning a visual discrimination-reversal learning task, a finding also observed in monkeys.

Postnatal oral exposure of primates to PCBs can result in long-term behavioral dysfunctions. In monkeys, oral exposure from birth to 20 weeks of age to 7.5 µg/kg/day of a PCB mixture, representative of the PCB residues generally found in human breast milk samples, also results in significant impairment in discrimination-reversal learning activities (Rice, 1997).

3.6. MECHANISMS OF TOXICITY

The most reliable and consistent symptom of TCDD toxicity among all experimental animals is 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.

Delayed expression of TCDD-induced toxic responses, including lethality, suggests that these toxic responses may not be the result of a direct insult by the parent compound (Mukerjee, 1998; Rozman, 1999). Progressive hypoglycemia from feed refusal and reduced gluconeogenesis seems to be the ultimate cause of TCDD-induced lethality (Gorski et al., 1990). In the L-E strain rat, reduced gluconeogenesis, indicated by decreased PEPCK activity, has been suggested to contribute to the acute toxicity of TCDD (Fan and Rozman, 1994; Viluksela et al., 1999). One of the major causes of TCDD-induced lethality also is dose-dependent reduction of tryptophan 2,3-dioxygenase (TdO) activity (Fan and Rozman, 1994; Stall et al., 1993), indicating

that subtle differences in the regulation of intermediary metabolism may be responsible for strain differences in the susceptibility of rats to TCDD (Fan and Rozman, 1994).

There have been significant advances in understanding the cause of TCDD-induced voluntary feed refusal. The neurotransmitter 5-hydroxytryptamine (5-HT), or serotonin, controlled by the availability of the amino acid tryptophan (Carlsson and Lindquist, 1978), suppresses feed intake behavior (Leibowitz, 1993). TCDD increases the plasma level of free tryptophan in L-E rats but not H/W rats (Unkila et al., 1994a). Increase in brain tryptophan levels (Rozman et al., 1991; Unkila et al., 1994b) and 5-HT turnover are closely connected with changes in plasma tryptophan (Unkila et al., 1994b). In L-E and H/W rats, the potencies of dioxin congeners highly correlate with their ability to disrupt tryptophan homeostasis. The order of potency is: TCDD > 1,2,3,7,8-PeCDD > 1,2,3,4,7,8-HxCDD > 1,2,3,4,6,7,8-HpCD (Unkila et al., 1998). TCDD lethal dose exposure results in increased brain 5-HT synthesis in L-E rats (Unkila et al., 1993), whereas in resistant H/W rats no such increase of 5-HT occurs. The dose-related changes in plasma free tryptophan are closely associated with the severity of the wasting syndrome observed in L-E rats (Unkila et al., 1994b). Increased circulating tryptophan and rapid turnovers of tryptophan and 5-HT in the brain are associated with TCDD-induced reduced feed intake, wasting, and lethality (Rozman et al., 1991; Unkila et al., 1994b). However, tryptophan metabolism or carbohydrate homeostasis does not explain the wide interspecies differences in susceptibility to acute lethality encountered between guinea pigs (the most acutely susceptible species) and hamsters (the most resistant species) (Unkila et al., 1995).

Despite extensive research to elucidate the ultimate events underlying the toxic action of TCDD, definitive answers are not yet available. The toxicity of TCDD apparently depends on the fact that the four lateral positions of the molecule are occupied by chlorine, resulting in high-affinity binding to the AhR. Mechanisms of toxicity are discussed in detail in Chapter 2. TCDD toxicity involves many different types of symptoms, which vary from species to species and from tissue to tissue, both quantitatively and qualitatively. Age- and sex-related differences in sensitivity have also 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 shown 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 a high-affinity 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. The dose required to produce the effect in an Ah-nonresponsive strain, however, 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 the 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 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 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 not be applicable to other species (Gasiewicz and Rucci, 1984).

In a genetic-crossing experiment between L-E and H/W rats (Pohjanvirta, 1990), it was demonstrated that the F_1 offspring were as resistant to TCDD toxicity as the H/W rats (LD_{50}, >3,000 µg/kg). Further studies on the F_2 generation indicated that the distribution of resistant and susceptible phenotypes was consistent with inheritance regulated by 2 (possibly 3) autosomal genes displaying complete dominance, independent segregation, and an additive coeffect.

Despite enormous variability in the recorded LD_{50} values for the guinea pig, rat, mouse, rabbit, and hamster, the amount and physical properties of the hepatic and 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 among the chick embryo, the C3H/HeN mouse, and the SD rat, the ED_{50} 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 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.

Recent observations suggest that some of the TCDD-induced toxicity in mice require other modes of action, beyond AhR-mediated DNA transcription. For example, wasting syndrome, thymus involution, and loss of adipose tissue in c-src$^{+/+}$ mice are correlated to c-src kinase activation, which is physically linked to AhR. These TCDD-induced toxic effects are not induced in src$^{-/-}$ mice and are marginal in c-src$^{-/+}$ mice (Matsumura et al., 1997a,b). These toxic effects can also be prevented in c-src$^{+/+}$ mice pretreated with geldanamycin, a c-src kinase inhibitor (Enan et al., 1998a; Dunlap et al., 1999). Based on c-src deficiency not affecting TCDD induction of the cytochrome P-450 type 1A1 isozyme (CYP1A1), the gene activation pathway of TCDD's action through the AhR nuclear translocator (ARNT) gene appears to be independent of the phosphorylation pathway of TCDD toxic activities modulated through the c-src gene. Involvement of c-src kinase activation in TCDD-induced toxicity has also been observed in the guinea pig. Enan et al. (1998b) showed that male guinea pigs pretreated with the src-kinase inhibitor geldanamycin did not suffer TCDD wasting. These investigators obtained similar results with src-deficient mice. Treatment with estradiol also protected male guinea pigs from TCDD-induced wasting. Furthermore, a nuclear AhR complex is not required for one of the signal transduction pathways associated with TCDD-induced early response of the c-fos and junB genes (Puga et al., 1992; Hoffer et al., 1996).

A strong correlation between lack of AhR affinity and lack of acute TCDD toxicity has been demonstrated in the knockout AhR$^{-/-}$ mouse. No significant difference in short-term toxicity was observed between the vehicle control group and knockout homozygous AhR$^{-/-}$ mice receiving TCDD at 2,000 µg/kg bw. Postexposure effects at day 28 were limited to vascularities of the lung and scattered necrosis of hepatocytes in AhR$^{-/-}$ resistant mice. In contrast, lipid accumulation and inflammatory cell infiltration of the liver were seen in heterozygous AhR$^{+/-}$-susceptible mice at the much lower dose of 200 µg/kg TCDD (Fernandez-Salguero et al., 1996). Although some of the TCDD-induced toxicity of the liver and thymus are mediated by the AhR, the mechanism for vascularities of the lung and the scattered necrosis of the lung and liver in AhR knockout mice may involve alternative pathways. As proposed by Matsumura et al. (1997a, b), these toxicity pathways still require the AhR and associated cytosolic proteins (Enan and Matsumura, 1996), but not nuclear AhR and DNA transcription.

Mutation of the p53 tumor suppresser gene associated with certain cancers confers resistance to TCDD-induced acute toxicity. The DBA/2 mouse has a complex mutation in the promoter region of the Tryp53 locus (the p53 region of the mouse). Both homozygous and heterozygous Tryp53 knockout mouse types have a high spontaneous incidence of cancer (Harvey et al., 1993). Inhibition of hepatocellular proliferation due to acute TCDD exposure also increases expression of the hepatic tumor suppressor p53 gene associated with the cell cycle inhibitory protein in the Balb/c mouse (Rininger et al., 1997). Results from these studies support

the hypothesis, proposed by Blagosklonny (1997), that high levels of the tumor suppressor p53 protein that confer protection against cancer may also increase sensitivity to the acute toxicity of TCDD.

3.7. SUMMARY

Most of the toxicity data available for TCDD are from oral experiments in animals. Very few percutaneous and no inhalation exposure toxicity data are available in the literature. Animal data following oral exposure indicate that TCDD is one of the most toxic compounds known and that it produces a wide spectrum of toxic effects.

There is a wide range of differences in sensitivity to TCDD lethality. The male guinea pig is the most sensitive, with an oral LD_{50} value of 0.6 µg/kg (Schwetz et al., 1973), and the male hamster the least sensitive, with an LD_{50} value of 5,051 µg/kg (Henck et al., 1981). This difference in sensitivity is more than 8,000-fold. The mink seems to be the second most sensitive to lethality, with an oral LD_{50} dose of 4.2 µg/kg for the male (Hochstein et al., 1988) and an LC_{50} value of 0.26 µg/kg for the female (Hochstein et al., 1998). The oral LD_{50} value for the male rat is 22 µg/kg (Schwetz et al., 1973). The rabbit LD_{50} value is 115 µg/kg (Schwetz et al., 1973). Unlike most toxic chemicals, the lethality of TCDD is delayed, with time to death being species and strain specific. Single lethal dose exposure results in death within 7-50 days and is generally associated with a wasting syndrome involving progressive loss of up to 50% body weight and eventual death without any clear or identifiable lethal pathological lesions. The characteristic signs and symptoms of lethal toxicity by TCDD are severe weight loss and thymic atrophy.

At least for acute exposure, the TCDD-induced toxicity appears to depend on the total dose administered over a given time, either through a single treatment or a limited number of multiple treatments. One of the consistent signs of TCDD toxicity in most species is thymic atrophy. Other toxic effects include hyperplasia or atrophy of the spleen, testes, or ovaries, bone marrow depletion, and systemic hemorrhage. Severe chloracne is one of the signs of TCDD exposure in people (Crow, 1978). Similar lesions or precursor lesions can be induced by TCDD in cattle (McConnell et al., 1980), rhesus monkeys (Norback and Allen, 1973; McConnell et al., 1978b), rabbits (Schwetz et al., 1973), and hairless mice (Knutson and Poland, 1982; Puhvel et al., 1982). The liver is extremely sensitive to TCDD toxicity in all animals, regardless of duration of exposure. The degree of severity of liver toxicity seems to be species-specific. Morphological alterations in liver toxicity are less severe in the guinea pig, the most sensitive species to lethality, than other species. The hamster, the most resistant species for lethality, shows liver lesions after a prolonged period of chronic exposure to nonlethal doses. Toxic effects include liver weight changes, fatty liver, impaired liver function characterized by increased microsomal monooxygenase, SGOT and SGPT activities, porphyrin accumulation,

impaired membrane function, hyperbilirubinemia, hypercholesterolemia, and hyperproteinemia. Severe episodes of toxic hepatitis have been observed in rats and mice. Various physiological equilibrium processes, such as vitamin A storage, plasma membrane functions, and the formation of keratin and cell differentiation, are affected by TCDD exposure. Pericardial and peritoneal edema resulting in death occur in chickens (Firestone, 1973). Systemic edema has also been observed in monkeys (Norback and Allen, 1973) and mice (Vos et al., 1974).

Sensitivity to TCDD toxicity segregates with the Ah locus. The potency of other congeners to induce lethality correlates with their ability to bind to the Ah receptor. Other congeners are less toxic than 2,3,7,8-TCDD. The lateral 2,3,7, and 8 position of the dioxin molecule must be chlorinated (or halogenated) to induce the greatest toxicity. The addition of chlorine atoms reduces toxicity. Increased microsomal AHH and EROD activities are markers for CYP1A1 (discussed in Chapter 2) and are associated — not necessarily causally — with the systemic toxicity of PCDDs, PCDFs, and coplaner PCBs.

Except for 2,3,7,8-TCDD and a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD, there are no long-term chronic bioassay data for PCDD/PCDF congeners that can be used for assessing the chronic risk. In the absence of any long term chronic toxicity bioassay data, enzymatic activity and other short-term in vivo and in vitro data are used in developing a toxicity ranking scheme (see Chapter 9).

One of the possible mechanisms by which TCDD and related compounds interfere with normal endocrine function is the ability to disrupt natural hormones. Hirsutism and diminished libido caused by TCDD seems to be due to its endocrine disruptive activities. A single oral dose of 20 µg/kg to rats can reduce serum testosterone levels (Nienstedt et al., 1979). Catabolism of exogenous estrone in TCDD-pretreated, ovariectomized rats is decreased (Shiverick and Muther, 1983). Alterations in hormonal levels by TCDD and its antiestrogenic action are discussed in Chapter 5. TCDD tumor promotion activity in liver carcinogenesis can be prevented in ovariectomized rats, indicating that estrogen status influences TCDD toxicity (Lucier et al., 1991). Animals exposed to TCDD and related compounds in utero or as infants exhibit varying degrees of behavioral disorders. These disorders resemble those seen in infants exposed to agents resulting in thyroid hormone deficiencies in utero or in infancy. Thyroidectomy of animals resulted in partial protection from TCDD-induced wasting syndrome and immunotoxicity, suggesting possible involvement of thyroid function in the manifestation of pathological conditions (Bastomsky, 1977; Rozman et al., 1984; Pazdernik and Rozman, 1985). Monkeys exposed for 4 years to TCDD at low ppt levels and studied for an additional 7-10 years have been reported to develop endometriosis (Rier et al., 1993). Estrogen, glucocorticoid, prolactin, insulin, gastrin, melatonin, and other hormones are affected by TCDD either by its

activity on the hormone or receptor. Further studies are needed to elucidate the mechanism of TCDD-induced endocrine disruptive activities.

The subchronic NOAEL for porphyria in female SD rats is estimated to be 0.01 µg/kg/week (= 0.001 µg/kg/day = 1 ng/kg/day) (Goldstein et al., 1982). A chronic NOAEL of 1 ng/kg/day for hepatotoxicity is estimated for SD rats from a 2-year chronic study (Kociba et al., 1978). In addition to liver toxicity, chronic exposure has been found to be associated with amyloidosis and dermatitis in Swiss mice (Toth et al., 1979). From this study, a LOAEL of 1 ng/kg/day for amyloidosis and dermatitis has been estimated for mice. Chronic exposure to 1.5 ng/kg/day in diet results in hair loss, edema, and pancytopenia in monkeys (Allen et al., 1977; Schantz et al., 1979). The lowest doses that have been demonstrated to elicit various biological responses in certain animals are compiled in Table 3-4.

Single-dose acute exposures to CDDs yield a large area under the curve (AUC) because of their long half-life and thus may be viewed as subchronic exposures. Subchronic/chronic exposures yield similar toxicity profiles to an acute exposure when similar total cumulative doses are administered. When corrected for excretion, depending on half-life, the corrected cumulative subchronic/chronic exposure doses seem to be analogous to the acute exposure doses. Similar data are available for 2,3,7,8-TCDF (Ioannou et al., 1983).

It is evident, from the complex picture evolving from the data outlined above, that TCDD elicits a variety of toxic responses following both short-term and long-term exposure. It is also clearly evident that there are very large differences in the sensitivity to specific TCDD-induced toxicities among various species and strains. This conclusion is valid for the severity of effects of almost all the responses studied. Qualitatively, however, there seems to be fairly good agreement among the types of responses that can be observed. For example, almost all responses can be produced in every species and strain if the right dose is chosen. Tissues or cell lines from humans and animals seem to respond to dioxin at similar exposure levels and in identical ways in regard to CYP1A1 activity, cytotoxicity, and inhibition of cell proliferation (DeVito et al., 1995). In highly sensitive species (e.g., the guinea pig), lethality may prevent some responses from occurring. Our present knowledge rules out enzyme induction as such, as the proximate 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 lethality. The available data indicate an involvement of TCDD in processes regulating cellular differentiation and proliferation, as well as those controlling endocrine 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 and species. The overwhelming number of toxic responses to TCDD, including lethality, typically show a delay in their appearance. This supports the assumption that these responses are not the result of a direct insult

from the compound. A lethal dose of TCDD in rats increases the neurotransmitter 5HT, controlled by increased levels of tryptophan in plasma and brain, suppresses voluntary feed consumption resulting in wasting syndrome, and leads eventually to mortality a few weeks postexposure. The induction of hepatic cytochrome P-450-dependent monooxygenases (such as CYP1A1) is one of the hallmarks of TCDD exposure. This effect has been demonstrated to be mediated through interaction with a specific protein called the Ah receptor. This process involves the binding of TCDD to the receptor, followed by the binding of the receptor-ligand complex to DNA recognition sites. This leads to the expression of specific genes and translation of their protein products, which then mediate their biological effects. As discussed in detail in Chapter 2, the mechanisms of action for enzyme induction are understood mainly at the molecular level, where the Ah receptor and its genetic regulation is clearly an important mechanistic step. Very little, however, is known about the mechanisms of the middle and high dose responses at the molecular level.

Studies in congenic mice that are relatively Ah-responsive or Ah-nonresponsive have demonstrated that the majority of TCDD-induced toxic responses segregate with the Ah locus. However, the number and affinity of Ah receptor expressed in most laboratory species and strains are rather comparable. The Ah receptor is thus unlikely to be the only determinant of TCDD-induced toxicity. Rather, it has to be assumed that species and strain differences are confined to the latter parts of the receptor-mediated chain of events (e.g., binding of the receptor-ligand complex to DNA and the subsequent expression of specific genes). In some cases, the binding affinity of the Ah receptor is different or defective. Some of the responses may be secondary in the sense that they are caused by the altered homeostasis of endogenous compounds, caused by TCDD-induced increased activities of various enzymes. An additional AhR-related pathway involving c-src kinase in the cytoplasm has been implicated in wasting syndrome, thymus atrophy, and loss of adipose tissue in mice.

Table 3-1. Acute lethality of TCDD to various species and substrains

Species/strain (sex)	Route	LD$_{50}$ (µg/kg)	Time of Death (days postexposure)	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., 1978a; 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., 1978b
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	>3,000	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 2,570 296	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. Acute lethality of TCDD to various species and substrains (continued)

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	1,157-5,051	2-47	50	NR	Henck et al., 1981
Hamster/Golden Syrian (male and female)	intraperitoneal	>3,000	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.

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 or metaplasia								
Gastric mucosa	++	0	+	0	0			0
Intestinal mucosa	+							++
Urinary tract	++	++	++	0	0			
Bile duct 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	+	±	++	+	++	+	+	±
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; Vos and Beems, 1971; Vos and Koeman, 1970.

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

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, 1,000, 5,000, 50,000, 500,000, 1,000,000 ng/kg	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, 1982
Monkey/Macaca mulatta	F/8	500 ng/kg	Continuous in the diet for 9 months	Extensive histopathology, hematology, and clinical chemistry	Allen et al., 1977

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	0.6 µg/kg, single oral dose	Lethality (single dose LD_{50})	Schwetz et al., 1973
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
Swiss mouse	1 ng/kg-day for 1 year	Amyloidosis and dermatitis	Toth et al., 1979
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., 1979
Sprague-Dawley rat	10 ng/kg-day for 2 years in feed	Porphyrin metabolism	Kociba et al., 1978

[a]0.6 ng/kg = no effect level.

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