OHEA-I-486e
March 1994
Internal Review Draft

Chapter 5. Developmental and Reproductive Toxicity

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

NOTICE

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

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

DISCLAIMER

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

5. DEVELOPMENTAL AND REPRODUCTIVE TOXICITY

5.1. INTRODUCTION

The potential for dioxins and related compounds to cause reproductive and developmental toxicity has been recognized for many years. Recent laboratory studies have broadened our knowledge in this area and suggest that altered development may be among the most sensitive TCDD endpoints. This chapter reviews much of the literature on dioxin's developmental and reproductive toxicity but is not intended to be exhaustive. Special emphasis is placed on that part of the database that has accumulated since the last major EPA review of this topic (Kimmel, 1988). In addition, the database is viewed in light of the Ah receptor model of TCDD action that is being examined for its applicability in the current EPA risk assessment.

To focus the analysis of the database, the chapter is divided into developmental toxicity and male and female reproductive toxicity. The authors recognize the interrelatedness of developmental and reproductive events at all levels of biological complexity. Therefore, the reader should not view the chapter subheadings within each of these divisions as defining discrete endpoints that are exclusive of other endpoints. For example, the effects of TCDD on circulating levels of sex hormones or on responsiveness to sex hormones may be translated into reproductive dysfunction if exposure occurs in adulthood or abnormal development of sexual behavior if exposure occurs perinatally. Likewise, even though organ structure and growth are considered separate manifestations in developmental toxicity that are associated with perinatal exposure to TCDD, the normal development of an organ is dependent on normal growth processes, and inhibiting perinatal growth can significantly disrupt the structural integrity of an organ system.

2,3,7,8-TCDD is one of 75 possible CDD congeners. It is one of the most potent of the CDDs, BDDs, CDFs, BDFs, PCBs, and PBBs and as such serves as the prototype congener for investigating the toxicity elicited by these classes of chemicals. Developmental and reproductive toxicity is generally believed to be caused by the parent compound; there is

no evidence that TCDD metabolites are involved. The toxic potency of TCDD is due to the number and position of chlorine substitutions on the dibenzo-*p*-dioxin molecule. CDD congeners with decreased lateral (2, 3, 7, and 8) or increased nonlateral chlorine and bromine substituents are less potent than TCDD (Safe, 1990); however, most of these congeners will produce toxicity, and the pattern of responses within animals of the same species, strain, sex, and age will generally be similar to that of TCDD (McConnell and Moore, 1979; Poland and Knutson, 1982). PCB congeners with zero or one *ortho* chlorines, two *para* chlorines, and at least two *meta* chlorines can assume a coplanar conformation sterically similar to TCDD and also produce a pattern of toxic responses similar to that of TCDD. In contrast, PCB congeners with two or more *ortho* chlorines cannot assume a coplanar conformation and do not resemble TCDD in toxicity (Poland and Knutson, 1982; Safe, 1990).

CDD and CDF congeners chlorinated in the lateral positions, as compared with those lacking chlorines in the 2, 3, 7, and 8 positions, are preferentially bioaccumulated by fish, reptiles, birds, and mammals (Stalling et al., 1983; Cook et al., 1991; U.S. EPA, 1991). Furthermore, coplanar PCBs and/or monoortho-chlorine-substituted analogs of the coplanar PCBs bioaccumulate in fish, wildlife, and humans (Tanabe, 1988; Kannan et al., 1988; Mac et al., 1988; Kubiak et al., 1989; Smith et al., 1990). This is of concern because combined effects of the lateral-substituted CDD, BDD, CDF, BDF, PCB, and PBB congeners acting through an Ah receptor mechanism have the potential of decreasing feral fish and wildlife populations secondary to developmental and reproductive toxicity (Gilbertson, 1989; Walker and Peterson, 1991; Walker et al., 1991; Cook et al., 1991). Humans are not exempt from the developmental and reproductive effects of complex halogenated aromatic hydrocarbon mixtures. Such mixtures that contain both TCDD-like congeners and non-TCDD-like congeners have been implicated in causing developmental and reproductive toxicity in the Yusho and Yu-Cheng poisoning incidents in Japan and Taiwan (Kuratsune, 1989; Hsu et al., 1985; Rogan, 1989). Thus, exposure to TCDD-like congeners is a health concern for

humans as well as for domestic animals, fish, and wildlife, although the relative contributions of TCDD-like and non-TCDD-like congeners are not known in some exposure situations.

A mechanism of action that CDD, BDD, CDF, BDF, PCB, and PBB congeners substituted in the lateral positions have in common is that they bind to the Ah receptor, which then binds to a translocating protein that carries the activated TCDD receptor complex into the nucleus. These activated TCDD receptor complexes bind to specific sequences of DNA referred to as dioxin-responsive enhancers (DREs), resulting in alterations in gene transcription. There is evidence that this Ah receptor mechanism, explained in detail in an earlier chapter, may be involved in the antiestrogenic action of TCDD and in its ability to produce the structural malformations of cleft palate and hydronephrosis in mice. However, its role in producing other signs of developmental and reproductive toxicity is not firmly established, leaving open the possibility that some TCDD effects are not Ah receptor mediated.

5.2. DEVELOPMENTAL TOXICITY

The manifestations of developmental toxicity from exposure to TCDD have been divided into three categories for convenience in assessing the database with respect to an Ah receptor-mediated response. These categories include death/growth/clinical signs, structural malformations, and functional alterations. Exposure-related effects on death/growth/clinical signs are described for fish, birds, laboratory mammals, and humans along with structure-activity results that are consistent with, but do not prove, an Ah receptor-mediated mechanism. Structural malformations, particularly cleft palate formation and hydronephrosis, occur in mice. In other mammalian species, however, postnatal functional alterations, some of which may be irreversible, are the most sensitive adverse developmental effects of TCDD-like congeners. These include effects on the male and female reproductive systems in rats and object learning behavior in monkeys.

5.2.1. Death/Growth/Clinical Signs

5.2.1.1. *Fish*

Early life stages of fish appear to be more sensitive to TCDD-induced mortality than adults. This is suggested by the LD_{50} of TCDD in rainbow trout sac fry (0.4 μg/kg egg weight) being 25 times less than that in juvenile rainbow trout (10 μg/kg body weight) (Walker and Peterson, 1991; Kleeman et al., 1988). The significance of this finding is that early life stage mortality caused by high concentrations of TCDD-like congeners in fish eggs may pose the greatest risk to feral fish populations (Walker and Peterson, 1991; Cook et al., 1991). Cooper (1989) reviewed the developmental toxicity of CDDs and CDFs in fish, and Cook et al. (1991) discussed components of an aquatic ecological risk assessment for TCDD in fish. The reader is referred to this literature for more indepth coverage than is presented here.

TCDD is directly toxic to early life stages of fish. This has been demonstrated for Japanese medaka, pike, rainbow trout, and lake trout exposed as fertilized eggs to graded concentrations of waterborne TCDD. In these species, TCDD causes an overt toxicity syndrome characterized by edema, hemorrhages, and arrested growth and development culminating in death (Helder, 1980, 1981; Wisk and Cooper, 1990a; Spitsbergen et al., 1991; Walker et al., 1991; Walker and Peterson, 1991). Histopathologic evaluation of lake trout embryos and sac fry has shown this syndrome to be essentially identical to that of blue sac disease (Helder, 1981; Spitsbergen et al., 1991). Following egg exposure to TCDD, signs of toxicity are not detected in medaka until after the liver rudiment forms (Wisk and Cooper, 1990a), and in lake trout toxicity is first detected ~ 1 week prior to hatching but becomes fully manifest during the sac fry stage (Spitsbergen et al., 1991; Walker et al., 1991). Among all fish species investigated thus far, lake trout are the most sensitive to TCDD developmental toxicity. Following exposure of fertilized lake trout eggs to graded waterborne concentrations of TCDD, the NOAEL for sac fry mortality is 34 pg TCDD/g egg, the LOAEL is 55 pg TCDD/g egg, and the egg TCDD concentration that causes 50 percent mortality above control at swim up (LD_{50}) is 65 pg TCDD/g egg (Walker et al.,

1991). Thus, TCDD is a potent developmental toxicant in fish and the effect is not secondary to maternal toxicity.

The Ah receptor has not been identified in early life stages of fish; however, it is assumed to be present because PCBs induce hepatic cytochrome P-4501A1 in lake trout and brook trout embryos and fry (Binder and Stegeman, 1983; Binder and Lech, 1984). The Ah receptor has been identified in adult rainbow trout liver (Heilmann et al., 1988) and in a rainbow trout hepatoma-cell line (Lorenzen and Okey, 1990). CDD and CDF congeners that are approximate isostereomers of TCDD produce essentially the same pattern of toxic responses as TCDD in early life stages of medaka and rainbow trout, suggesting that they may act through a common mechanism (Wisk and Cooper, 1990b; Walker and Peterson, 1991). Also, in rainbow trout their potencies relative to TCDD (i.e., toxic equivalency factors, TEFs) for causing early life stage mortality (TCDD LD_{50}/congener LD_{50}) are in the same range as those proposed for human health risk assessment based on a diverse spectrum of acute and subchronic toxicity tests in mammalian species (Safe, 1990; Walker and Peterson, 1991). However, for the coplanar PCBs and monoortho-chlorinated analogs of the coplanar PCBs, TEFs based on early life stage mortality in rainbow trout are 1/14 to 1/80 less (Walker and Peterson, 1991) than the TEFs proposed for risk assessment (Safe, 1990).

5.2.1.2. *Birds*

Bird embryos are also more sensitive to TCDD toxicity than adults. The LD_{50} of TCDD in the chicken embryo (0.25 μg/kg egg weight) is 100 to 200 times less than the TCDD dose that causes mortality in adult chickens (25-50 μg/kg body weight) (Greig et al., 1973; Allred and Strange, 1977). The LD_{50} of TCDD injected into fertilized ring-necked pheasant eggs (1.1-1.8 μg/kg egg weight) is 14 to 23 times less than the TCDD dose that causes 75 percent mortality in ring-necked hen pheasants (25 μg/kg body weight) (Nosek et al., 1993).

Among bird species, most developmental toxicity research has been done on chickens. Injection of TCDD or its approximate isostereomers into fertilized chicken eggs causes a

toxicity syndrome in the embryo characterized by pericardial and subcutaneous edema, liver lesions, inhibition of lymphoid development in the thymus and bursa of Fabricius, microphthalmia, beak deformities, cardiovascular malformations, and mortality (Cheung et al., 1981a,b; Brunstrom and Darnerud, 1983; Rifkind et al., 1985; Brunstrom and Lund, 1988; Brunstrom and Andersson, 1988; Nikolaidis et al., 1988a,b). On the other hand, injection of a coplanar PCB into fertilized turkey eggs at a dose high enough to cause microphthalmia, beak deformities, and embryo mortality did not produce liver lesions, edema, or thymic hypoplasia, all hallmark signs of TCDD toxicity in the chicken embryo (Brunstrom and Lund, 1988). This disparity in signs of TCDD embryotoxicity among bird species is not unique to the turkey and chicken. In fertilized eggs of ring-necked pheasants and eastern bluebirds, injection of TCDD produces embryo mortality, but all of the other signs of toxicity seen in the chicken embryo are absent, including cardiovascular malformations (Thiel et al., 1988; Martin et al., 1989; Nosek et al., 1993). Thus, in bird embryos the signs of toxicity elicited by TCDD and its approximate isostereomers are highly species dependent; the only toxic effect common to all bird species is embryo mortality.

There is evidence in chicken embryos that the Ah receptor may be involved in producing developmental toxicity. The Ah receptor has been detected in chicken embryos (Denison et al., 1986; Brunstrom and Lund, 1988) and the rank order potency of PCB congeners for producing chicken embryo mortality (3,3′,4,4′,5-PCB > 3,3′,4,4′-TCB > 3,3′,4,4′,5,5′-HCB > 2,3,3′,4,4′-PCB > 2,3,4,4′,5-PCB, with 2,2′,4,5′-TCB, 2,2′,4,4′,5,5′-HCB, and 2,2′,3,3′,6,6′-HCB being inactive) is similar to that for a classic Ah receptor-mediated response in the chicken embryo cytochrome P-4501A1 induction (Rifkind et al., 1985; Brunstrom and Andersson, 1988; Brunstrom, 1989). However, although induction of cytochrome P-4501A1 and toxicity may both be part of a pleiotropic response linked to the Ah receptor, they are not otherwise causally related. This is demonstrated by the nonsteroidal anti-inflammatory drug benoxoprofen that suppresses 3,3′,4,4′-TCB-induced toxicity in the chicken embryo without altering its ability to induce microsomal enzyme activity (Rifkind and Muschick, 1983). Also, for 3,3′,4,4′-TCB, 3,3′,4,4′,5,5′-HCB, and

TCDD there is a marked dissociation of the dose-response relationship for lethality and enzyme induction in the chicken embryo (Rifkind et al., 1985).

A decreased activity of uroporphyrinogen decarboxylase (URO-D) and an increased accumulation of uroporphyrins are effects that are readily produced by exposure of cultured chicken embryo liver cells to TCDD, 3,3′,4,4′-TCB, and other PCBs (Sinclair et al., 1984; Marks, 1985; Lambrecht et al., 1988). Coplanar PCB congeners are more potent inhibitors of URO-D activity in cultured chicken embryo liver cells than are noncoplanar PCB congeners (Sassa et al., 1986), suggesting an Ah receptor-mediated mechanism. Unlike the results in cultured cells, however, a lethal dose of TCDD (6 nmol/egg) does not affect URO-D activity or cause an increased accumulation of uroporphyrins in chicken embryos (Rifkind et al., 1985). Thus, TCDD-induced lethality in chicken embryos is not associated with the effects of TCDD on URO-D activity, even though a decrease in URO-D activity might be expected to occur if a sufficient dose of TCDD could be reached without being lethal.

The chicken embryo heart is a target organ for TCDD and other halogenated aromatic hydrocarbons that act by an Ah receptor mechanism. The classic sign of chick embryo toxicity involving the heart is pericardial edema. However, TCDD has other effects on the chick embryo heart that are less well known. These include its ability to produce cardiovascular malformations and to increase cardiac release of arachidonic acid metabolites. When fertilized chicken eggs are injected with graded doses of TCDD, cardiovascular malformations are produced including ventricular septal defects, aortic arch anomalies, and conotruncal malformations. Approximately 1.6 pmol TCDD/egg (9 ng/kg egg, assuming a 55 g egg weight) causes cardiovascular malformations in 46 percent of treated embryos versus 29 percent of control embryos (Cheung et al., 1981a,b). The cardiovascular malformation response may be unique to the chicken embryo because in fertilized ring-necked pheasant and eastern bluebird eggs injected with TCDD the incidence of such malformations is not increased (Thiel et al., 1988; Martin et al., 1989; Nosek et al., 1993).

In the chicken embryo heart, arachidonic acid metabolism is stimulated by TCDD, resulting in increased formation of prostaglandins (Quilley and Rifkind, 1986). Dose-

response relationships for the release of immunoreactive PGE_2, $PGF_{2\alpha}$, and TxB_2 from chick embryonic heart are biphasic with an apparent maximally effective dose of 100 pmol TCDD/egg. When the egg TCDD dose is further increased, release of these prostaglandins tends to decline towards levels in control hearts. Biphasic dose response curves for cardiac PGE_2 release also were obtained with 3,3′,4,4′-TCB and 3,3′,4,4′,5,5′-HCB (Quilley and Rifkind, 1986). The thymus and bursa of Fabricius are other TCDD target organs in the chicken embryo. TCDD, 3,3′,4,4′-TCB, and 3,3′,4,4′-TCAOB cause dose-related decreases in the lymphoid development of both of these immune system organs (Nikolaidis et al., 1988a,b, 1990). Cultured thymus anlage from chick embryos are 100 times more sensitive to TCDD's inhibitory effect on lymphoid development than cultured thymus anlage from turkey and duck embryos (Nikolaidis et al., 1988a). This suggests that the reason thymic atrophy was not seen in turkey embryos at egg doses of 3,3′,4,4′-TCB that were overtly toxic (Brunstrom and Lund, 1988) was not because the turkey embryo thymus was incapable of responding to 3,3′,4,4′-TCB. Rather, turkey embryos appear to be more sensitive to the lethal than to the immunotoxic effect of this coplanar PCB.

Within the same bird species, the signs of developmental toxicity elicited by TCDD and its approximate isostereomers are similar. In the chicken embryo, TCDD, 3,3′,4,4′,5-PCB, 3,3′,4,4′-TCB, and 3,3′,4,4′,5,5′-HCB all cause pericardial and subcutaneous edema, liver lesions, micropthalmia, beak deformities, and mortality, and TCDD, 3,3′,4,4′-TCB, and 3,3′,4,4′-TCAOB inhibit lymphoid development (Cheung et al., 1981a; Brunstrom and Andersson, 1988; Nikolaidis et al., 1988a,b). In pheasant embryos, an altogether different pattern of responses is seen. Nevertheless, the TCDD-like congeners injected into fertilized pheasant eggs, TCDD and 3,3′,4,4′-TCB, produce the same pheasant embryo-specific pattern. This pattern consists of embryo mortality in the absence of edema, liver lesions, thymic hypoplasia, and structural malformations (Brunstrom and Reutergardh, 1986; Nosek et al., 1993).

The lethal potency of TCDD and its approximate isostereomers in embryos of different bird species varies widely. The chicken embryo is an outlier in that it is by far the

most sensitive of all bird species to TCDD. Turkey, ring-necked pheasant, mallard duck, domestic duck, domestic goose, golden-eye, herring gull, black-headed gull, and eastern bluebird embryos are considerably less sensitive to the embryo lethal effect of TCDD and TCDD-like congeners (Brunstrom and Reutergardh, 1986; Brunstrom and Lund, 1988; Thiel et al., 1988; Martin et al., 1989; Elliott et al., 1989; Nosek et al., 1993). TCDD is 4 to 7 times more potent in causing embryo mortality in chicken than pheasant embryos, and 3,3′,4,4′-TCB is 20 to 100 times more potent in chicken than turkey embryos (Allred and Strange, 1977; Brunstrom and Lund, 1988; Nosek et al., 1989). In chicken embryos, an egg dose of 3,3′,4,4′-TCB of 4 μg/kg increased embryo mortality, whereas an egg dose of 100 μg/kg of the same coplanar PCB had no embryotoxic effect in pheasants and mallard ducks and a dose of 1,000 μg/kg egg had no effect on embryo mortality in domestic ducks, domestic geese, golden eyes, herring gulls, and black-headed gulls (Brunstrom, 1988; Brunstrom and Reutergardh, 1986). In contrast to the above species differences, the potency of 3,3′,4,4′-TCB in causing embryo mortality among different strains of chickens is quite similar, with the LD_{50} in six different strains varying less than fourfold (Brunstrom, 1988).

Graded doses of TCDD have been administered to fertilized eastern bluebird and ring-necked pheasant eggs for the purpose of determining an LOAEL and NOAEL for embryotoxicity. Mortality was the most sensitive embryotoxic effect in both species. For eastern bluebirds, the LOAEL was 10,000 pg TCDD/g egg and the NOAEL was 1,000 pg TCDD/g egg (Martin et al., 1989). For ring-necked pheasants, the LOAEL was 1,000 pg TCDD/g egg and the NOAEL was 100 pg TCDD/g egg. The LD_{50} for embryo mortality in the ring-necked pheasant is 1,354 pg TCDD/g egg when the dose is injected into the egg albumin and 2,182 pg TCDD/g egg when the dose is injected into the egg yolk (Nosek et al., 1993). In contrast, for chickens the LD_{50} for embryo mortality is 240 pg TCDD/g egg (Allred and Strange, 1977).

5.2.1.3. *Laboratory Mammals*

When exposed to TCDD during adulthood, laboratory mammals display wide differences in the LD_{50} of TCDD. It is interesting to note, however, that when exposure occurs during prenatal development, the potency of TCDD tends to be more similar across species. The LD_{50} of TCDD in adult hamsters, 1,157 to 5,051 μg/kg, makes adult hamsters three orders of magnitude more resistant to TCDD-induced lethality than are adult guinea pigs (Olson et al., 1980; Henck et al., 1981). Yet, a maternal dose of 18 μg TCDD/kg can increase the incidence of prenatal mortality in the hamster embryo/fetus. Since this dose is only twelvefold larger than the dose (1.5 μg TCDD/kg) that increases the incidence of prenatal mortality in the guinea pig, the hamster embryo/fetus approaches other rodent species in its sensitivity to TCDD-induced lethality (Olson and McGarrigle, 1990, 1991). Thus, the magnitude of the species differences in lethal potency of TCDD is affected by the timing of TCDD exposure during the life history of the animal.

Exposure to TCDD during pregnancy causes prenatal mortality in the monkey, guinea pig, rabbit, rat, hamster, and mouse (Table 5-1). Given a particular dosage regimen, the response is dose related and there appear to be species and/or strain differences in susceptibility to TCDD-induced prenatal mortality. The rank order of susceptibility from the most sensitive to least sensitive species would appear to be monkey = guinea pig > rabbit = rat = hamster > mouse. However, an important caveat must be applied to the information presented in Table 5-1; i.e., that the time period during which exposure of the embryo/fetus to TCDD occurs is just as important a determinant of prenatal mortality as is the dose of TCDD administered. This point will be illustrated in the text that follows when prenatal mortality is described for different strains of mice.

It is important to note that the concept of a critical time period for exposure makes the analysis of lethality data in the embryo/fetus qualitatively different from that which might be applied to similar data in adult animals. For example, a common dosing regimen used in mice, rats, and rabbits (Table 5-1) is to administer 10 daily doses of TCDD to the pregnant dam on days ~6 to 15 of gestation. This dosing regimen is expected to cover the critical

TABLE 5-1
Relationship Between Maternal Toxicity and Prenatal Mortality in Laboratory Mammals Exposed to TCDD During Gestation

Species/strain	Daily TCDD dose (μg/kg/day)	Cumulative TCDD dose (μg/kg)	Overt maternal toxicity[a]	Percent prenatal mortality[b]	Reference
Monkey/rhesus		0[c]	-	25	McNulty, 1984
		0.2	-	25	
		1	+[d]	81	
		5	+[d]	100	
Guinea pig/Hartley		0[e]	-	-	Olson and McGarrigle, 1991
		0.15	-	-	
		1.5	+	+	
Rabbit/New Zealand	0[f]	0	-	7	Giavini et al., 1982b
	0.1	1	-	12	
	0.25	2.5	+	42	
	0.5	5	+	22	
	1	10	+	100	
Rat/Wistar	0[f]	0	-	3	Khera and Ruddick, 1973
	0.125	1.25	-	1	
	0.25	2.5	-	2	
	0.5	5	-	9	
	1	10	±	8	
	1	10	+	36[g]	
	2	20	+	53[g]	
	4	40	+	100[g]	
Rat/Sprague-Dawley	0[f]	0	-	25	Sparschu et al., 1971
	0.03	0.3	-	21	
	0.125	1.25	-	15	
	0.5	5	+	41[g]	
	2	20	+	95[g]	
	8	80	+	100[g]	
Hamster/Golden Syrian		0[h]	-	-	Olson and McGarrigle, 1991
		1.5	-	-	
		3	-	-	
		6	-	-	
		18	-	58	
Mouse/CD-1	0[i]	0	-	7	Courtney, 1976
	25	250	-	6	
	50	500	-	13	
	100	1,000	-	14	
	200	2,000	+	87	
	400	4,000	+	97	

[a]Decreased body weight gain or marked edema compared with vehicle dosed controls. A (+) or (-) indicates the presence or absence of an effect.
[b]Percentage of absorptions plus late gestational deaths relative to all implantations. A (+) or (-) indicates the presence or absence of an effect.
[c]TCDD administered in a single or divided doses between gestational days 20 and 40.
[d]Effects include thickening and reddening of the eyelids, weight loss, dryness and granularity of the skin, loss of hair, and in some cases anemia, purpura, and bleeding from the nose and mouth.
[e]Single dose of TCDD administered on gestational day 14.
[f]TCDD administered daily on days 6 to 15 of gestation.
[g]Significant at $p < 0.05$.
[h]Single dose of TCDD administered on gestational days 7 or 9.
[i]TCDD administered daily on days 7 to 16 of gestation.

Source: Couture et al., 1990a.

period of early development that results in the greatest incidence of prenatal toxicity. In nearly all species of adult laboratory mammals, however, a single lethal dose of TCDD would be expected to produce a similar delayed-onset death regardless of the age of the adult animal. Susceptibility to TCDD-induced prenatal mortality, in contrast, may be greatly dependent on the age of the embryo/fetus. In this case, multiple doses of TCDD that cover this critical period might result in prenatal mortality, whereas a single dose might miss the critical time and not result in prenatal mortality.

The following paragraphs illustrate a type of analysis using an index of cumulative maternal dose similar to the type of analysis that might be applied to lethality data resulting from multiple dosing of adult animals. After presenting the results of applying this type of analysis to prenatal mortality data from different species, the caveat of critical time dependence will be applied to the data obtained by using different strains of mice. This will illustrate the importance of considering dosage regimen when evaluating prenatal mortality data that are available in the literature. In this case, a difference of 1 gestational day might be critically important. It turns out that the form of analysis using cumulative maternal dose may give the greatest possible degree of species variation. As such, different species may actually be more similar with respect to susceptibility to prenatal mortality than would be apparent from the results of this type of an analysis.

Using the cumulative dose data that are given in Table 5-1, there appears to be a tenfold to twentyfold difference in the fetolethal potency of TCDD when the monkey/guinea pig is compared with the rabbit/rat/hamster. In the CD-1 mouse treated with TCDD on gestational days 7 to 16, it appears that a daily dose of 200 μg TCDD/kg is required to significantly increase prenatal mortality. Given a ~5.5-day half-life of TCDD in the pregnant dam (Weber and Birnbaum, 1985), the pregnant CD-1 mouse would be exposed to a maximal accumulated dose, of ~1,200 μg TCDD/kg by the lowest dosage regimen that significantly increased prenatal mortality. Therefore, by using the index of cumulative dose, the CD-1 mouse would appear to be ~1,200-fold less sensitive than the monkey/guinea pig for TCDD-induced prenatal mortality. However, in NMRI mice administered TCDD only

on day 6 of gestation, prenatal mortality begins to increase after a single dose of 45 μg TCDD/kg (Neubert and Dillman, 1972). The NMRI embryo/fetus is less susceptible to TCDD-induced prenatal mortality when the TCDD is administered on later gestational days up to day 15. Thus, there appears to be only an approximate 45-fold difference between the monkey/guinea pig and the NMRI mouse when the NMRI embryo/fetus is exposed specifically on day 6. In C57BL/6 mice, prenatal mortality is significantly increased after a single maternal dose of 24 μg TCDD/kg given on gestational day 6 (Couture et al., 1990b). This mouse strain, therefore, is about twentyfold to thirtyfold less sensitive to TCDD-induced prenatal mortality than is the monkey/guinea pig when exposed specifically on day 6. As with the NMRI mouse, there was little or no increase in prenatal mortality for the C57BL/6 strain when TCDD was administered to the pregnant dam on gestational days 8, 10, 12, or 14.

Mammalian pregnancies (including the human) are characterized by critical periods or "windows" during which the embryo/fetus exhibits different susceptibilities and responses to chemical exposures. As the embryo matures into a fetus and a neonate capable of extrauterine life, the responses to chemical insult shift from a predominance of death and structural alterations to changes in growth and functional maturation. The susceptibility of any particular endpoint depends on the developmental state of that endpoint at the time of exposure. The embryo/fetus is constantly changing at all biological levels (e.g., cellular, tissue, organism), and the mechanisms of action, response, and repair of a particular endpoint at the time of exposure are the determinants of whether a response to a given exposure will result in a developmental alteration or not.

The concept of a critical window for TCDD-induced lethality in the embryo/fetus suggests an explanation for the apparent insensitivity of the CD-1 embryo/fetus exposed to cumulative doses of TCDD. It could very well be that the critical window for prenatal mortality in the mouse occurs on or before gestational day 6. If the embryo/fetus is not exposed to TCDD by gestational day 6, much larger doses of TCDD are required to produce prenatal mortality. Given that exposure of the pregnant CD-1 dams did not begin until

gestational day 7, this interpretation is consistent with the ability of a single 24 μg TCDD/kg dose to increase the incidence of prenatal mortality when administered to pregnant C57BL/6 mice on gestational day 6, but not when administered on gestational days 8, 10, 12, or 14 (Couture et al., 1990b). Similarly, Neubert and Dillman (1972) found that the largest increase in prenatal mortality occurred when a single dose of TCDD was given on gestational day 6 compared with prenatal mortality when the TCDD dose was administered on one of gestational days 7 to 15. In addition, this would suggest that the CD-1 embryo/fetus does not have quite the relative insensitivity to the lethal effects of TCDD compared with the embryo/fetus of other species that would be indicated by using cumulative maternal dose as the index of exposure.

It should be noted that the concept of a critical window for prenatal mortality could potentially alter all of the species comparisons made previously that were based on the cumulative maternal doses shown in Table 5-1. If this turned out to be the case, then the true differences between species with respect to their susceptibility to TCDD-induced prenatal mortality could be substantially less than those indicated by using the cumulative maternal dose. This, of course, would involve a comparison between species using only single doses of TCDD given during the critical time period for each species. At the present time, it is not possible to make such a comparison from the information available in the literature.

Similar to fish and birds, the mammalian embryo/fetus is more sensitive to the lethal action of TCDD than the adult. The maternal dose of TCDD that causes 58 percent fetal mortality in hamsters is 64 to 280 times less than the LD_{50} of TCDD in adult hamsters (Olson et al., 1980; Henck et al., 1981; Olson et al., 1990). In Sprague-Dawley rats, the cumulative maternal dose of TCDD that causes 41 percent prenatal mortality is 5 to 10 times less than the approximate LD_{50} of TCDD in adult rats of the same strain (Sparschu et al., 1971; Seefeld et al., 1984). In rhesus monkeys, the cumulative maternal TCDD dose that causes 81 percent prenatal mortality is 6 and 25 times less, respectively, than the lowest

TCDD dose reported to cause mortality in 1-year-old and adult rhesus monkeys (McNulty, 1977, 1985; Seefeld et al., 1979).

A general finding in all nonprimate laboratory mammals, with the possible exception of the hamster, is that TCDD-induced prenatal mortality is most commonly associated with maternal toxicity that is not severe enough to result in maternal lethality. This is seen in Table 5-1 for the guinea pig, rabbit, rat, and mouse. In each species, the dose-response relationship for maternal toxicity, indicated by decreased maternal weight gain and/or marked subcutaneous edema of the dam, is essentially the same as that for increased prenatal mortality. What this means is that there may be an association between the fetolethal effect of TCDD and maternal toxicity in all of these species. Even in the hamster, where maternal toxicity is far less severe, fetuses exhibit increases in neutrophilic metameylocytes and bands, and increases in leukocyte number and bands are also found in maternal blood (Olson and McGarrigle, 1991). More recently in mice, it has been shown that TCDD exposure causes rupture of the embryo-maternal vascular barrier, which results in hemorrhage of fetal blood into the maternal circulation (Khera, 1992). It is not known whether these extraembryonic hematologic changes are contributory to or coincidental with developmental toxicity in these species. However, their occurrence reinforces the concept that prenatal mortality can be associated with maternal toxicity.

In rhesus monkeys, on the other hand, fewer data are available to make the association between prenatal mortality and maternal toxicity. Although only small numbers of monkeys have been studied to date, the results following dietary exposure to 25 ppt TCDD (Bowman et al., 1989b; Schantz and Bowman, 1989) and 50 ppt TCDD (Allen et al., 1977, 1979; Barsotti et al., 1979; Schantz et al., 1979) before and during pregnancy suggest that TCDD-induced prenatal mortality can occur in monkeys in the absence of overt toxic effects on the mother (see Section 5.3.1). In other studies, developmental toxicity in monkeys exposed to a total cumulative maternal dose of 1 μg TCDD/kg administered during the first trimester indicated a high incidence of prenatal mortality (McNulty, 1984, 1985). However, maternal toxicity occurred in some but not all of the mothers exposed. In these

monkeys, 13 of 16 pregnancies resulted in prenatal mortality. Within 20 to 147 days after aborting, 8 of the 13 females that had aborted showed signs of maternal toxicity and three of these monkeys died. Thus, the remaining 5 of 13 instances of prenatal mortality apparently occurred in the absence of overt maternal toxicity. The results of these studies indicate that some levels of TCDD exposure can result in prenatal mortality in monkeys even though overt toxicity seems absent in the mother. As will be described (Section 5.3.1.1), however, only limited attention has been given to female reproductive toxicity in general and to the effects of maternal toxicity during pregnancy on fetal development in particular. Therefore, the relationship between maternal toxicity and prenatal mortality in the monkey is not well established. The integrity of the embryo-vascular barrier, for example, has not been evaluated after TCDD exposure.

Gestational exposure to TCDD produces a characteristic pattern of fetotoxic responses in most laboratory mammals consisting of thymic hypoplasia, subcutaneous edema, decreased fetal growth, and prenatal mortality. Added to these common fetotoxic effects are other effects of TCDD that are highly species-specific. Examples of the latter are cleft palate formation in the mouse and intestinal hemorrhage in the rat. Table 5-2 shows those maternal and fetal toxic responses that are produced by gestational exposure to TCDD in various species of laboratory mammals. In the mouse, hydronephrosis is the most sensitive effect of prenatal toxicity, followed by cleft palate formation and atrophy of the thymus at higher doses, and by subcutaneous edema and mortality at maternally toxic doses (Couture et al., 1990a; Courtney, 1976; Courtney and Moore, 1971; Neubert and Dillman, 1972). In the rat, TCDD prenatal toxicity is manifested by intestinal hemorrhage, subcutaneous edema, decreased fetal growth, and mortality (Sparschu et al., 1971; Khera and Ruddick, 1973). Structural abnormalities do occur in the rat but only at relatively large doses (Couture et al., 1990a). In the hamster fetus, hydronephrosis and renal congestion are the most sensitive effects, followed by subcutaneous edema and mortality at fetolethal doses (Olson and McGarrigle, 1991). In the rabbit, an increased incidence of extra ribs and prenatal mortality

TABLE 5-2
Developmental Toxicity Following Gestational Exposure to 2,3,7,8-TCDD

Species/strain	Daily dose[a]	Treatment days[b]	Sacrifice day[b]	Maternal effects[c]	Embryo/fetal effects[c]	Reference
Mice/C57BL/6N	0, 1, or 3 μg/kg	10, 10-13	18	NR	Increase in cleft palate and hydronephrosis	Moore et al., 1973
Mice/C57BL/6N	0, 12, 17, or 22 μg/kg	10	18	Increase in liver-to-body weight ratio	Increase in cleft palate and hydronephrosis	Weber et al., 1985
Mice/C57BL/6N	0, 3, or 12 μg/kg	11, 10-13	18	Increase in liver-to-body weight ratio	Increase in cleft palate and hydronephrosis	Birnbaum et al., 1985
Mice/C57BL/6N	0 or 3 μg/kg	10-13	18	Increase in liver-to-body weight ratio	Increase in hydronephrosis	Birnbaum et al., 1986
Mice/C57BL/6N	0, 6, 9, 12, 15, or 18 μg/kg	10, 12	18	Increase in liver-to-body weight ratio and weight gain	Increase in cleft palate and hydronephrosis	Birnbaum et al., 1989
Mice/C57BL/6J	0 or 3 μg/kg (subcutaneous)	6-15	18	Increase in liver-to-body weight ratio	Increase in cleft palate and kidney anomaly	Courtney and Moore, 1971
Mice/C57BL/6J	20 μg/kg	10	17	Increase in liver-to-body weight ratio	Increase in cleft palate, hydronephrosis, and fetal body weight	Haake et al., 1987
Mice/C57BL/6J	0, 0.5, 1, 2, or 4 μg/kg	6-15	18	Increase in liver-to-body weight ratio	Increase in cleft palate, hydronephrosis, and fetal body weight	Silkworth et al., 1989
Mice/NMR	0.3, 3, 4, 5, or 9 μg/kg	6-15	18	NR	Increase in cleft palate and fetal mortality; decrease in fetal body weight	Neubert and Dillman, 1972
Mice/CF-1	0, 0.001, 0.01, 0.1, or 1.3 μg/kg	6-15	NR	None	Increase in cleft palate and hydronephrosis	Smith et al., 1976
Mice/DBA	0 or 3 μg/kg (subcutaneous)	6-15	18	Increase in liver-to-body weight ratio	Increase in cleft palate and kidney anomaly	Courtney and Moore, 1971
Mice/DBA	0, 0.5, 2, 4, or 8 μg/kg	6-15	18	Increase in liver-to-body weight ratio; decrease in thymus-to-body weight ratio	Increase in cleft palate and hydronephrosis	Silkworth et al., 1989
Mice/CD-1	0, 1, or 3 μg/kg (subcutaneous)	6-15	17	None	Increase in cleft palate and kidney anomaly	Courtney and Moore, 1971

TABLE 5-2 (continued)

Species/strain	Daily dose[a]	Treatment days[b]	Sacrifice day[b]	Maternal effects[c]	Embryo/fetal effects[c]	Reference
Mice/CD-1	0, 25, 50, 100, 200, or 400 μg/kg	6-15	17	Increase in liver-to-body weight ratio	Increase in cleft palate, hydronephrosis, and fetal mortality	Courtney, 1976
Rats/CD	0 or 0.5 μg/kg 2 μg/kg (subcutaneous)	6-15 9-10 or 13-14	20	None at 0.5 μg/kg NR at 2 μg/kg	Increase in kidney anomaly	Courtney and Moore, 1971
Rats/Sprague-Dawley	0, 0.125, 0.5, or 2 μg/kg	0-2	20	Decrease in weight gain	Decrease in fetal body weight	Giavini et al., 1982a
Rats/Sprague-Dawley	0.03, 0.125, 0.5, 2, or 8 μg/kg	6-15	21	Decrease in weight gain; toxicity	Increase in fetal mortality, resorptions, edema, and gastrointestinal hemorrhage	Sparschu et al., 1971
Rats/Wistar	0, 0.125, 0.25, 0.5, 1, 2, 4, 8, or 16 μg/kg	5-14	21	Toxicity	Increase in fetal mortality, edema and gastrointestinal hemorrhage; decrease in fetal weight	Khera and Ruddick, 1973
Guinea pigs/Hartley	0, 0.15, or 1.5 μg/kg	14	58	Increase in mortality; toxicity	Increase in fetal mortality	Olson and McGarrigle, 1990
Hamsters/Golden Syrian	0, 1.5, 3, 6, or 18 μg/kg	7, 9	15	Increase in liver-to-body weight ratio	Increase in fetal mortality, hydronephrosis, and renal congestion; decrease in thymus size	Olson and McGarrigle, 1990
Rabbits/New Zealand	0, 0.1, 0.25, 0.5, or 1 μg/kg	6-15	28	Decrease in weight gain; toxicity	Increase in fetal mortality and resorptions; extra ribs	Giavini et al. 1982b
Monkeys/rhesus	0, 5, 25, 50, or 500 ppt 7 months before and during pregnancy	Chronic	--	Increase in mortality; toxicity	Increase in fetal mortality	Allen et al., 1979; Bowman et al., 1989
Monkeys/rhesus	0, 0.2[d], 1[d], 1[e], or 5[d] μg/kg	20-40	--	Increase in mortality; toxicity	Increase in fetal mortality	McNulty, 1985

[a]Oral exposure unless otherwise noted.
[b]All days adjusted to reflect plug day—gestation day 0.
[c]Effects reported are only those that were statistically significant.
[d]Cumulative dose divided into nine oral doses administered between days 20 and 40 of gestation, two to four monkeys/dose.
[e]Three animals given single oral dose, on either gestation days 25, 30, 35, or 40; 12 monkeys total.
NR = Not reported.

Source: Couture et al., 1990a.

is found (Giavini et al., 1982b), and in the guinea pig and rhesus monkey, prenatal mortality is seen (Olson and McGarrigle, 1991; McNulty, 1984).

5.2.1.4. *Structure-Activity Relationships in Laboratory Mammals*

The structure-activity relationship for developmental toxicity in laboratory mammals is generally similar to that for Ah receptor binding. Gestational treatment of rats with CDD congeners that do not bind the Ah receptor, 2-MCDD, 2,7-DCDD, 2,3-DCDD, or 1,2,3,4-TCDD, do not cause TCDD-like fetotoxic effects (Khera and Ruddick, 1973). On the other hand, hexachlorodibenzo-*p*-dioxin, which has intrinsic Ah receptor activity, produces fetotoxic responses in rats that are essentially identical to those of TCDD (Schwetz et al., 1973). Similarly, when administered to pregnant rhesus monkeys or CD-1 mice, PCB congeners that act by an Ah receptor-mediated mechanism, 3,3′,4,4′-TCB and 3,3′,4,4′,5,5′-HCB, cause the same type of fetotoxic effects as TCDD. In contrast, 4,4′-DCB, 3,3′,5,5′-TCB, 2,2′,4,4′,5,5′-HCB, 2,2′,4,4′,6,6′-HCB, and 2,2′,3,3′,5,5′-HCB, which have essentially no or a very weak affinity for the Ah receptor, do not produce a TCDD-like pattern of prenatal toxicity in mice (Marks and Staples, 1980; Marks et al., 1981, 1989; McNulty, 1985). Thus, most structure activity results for overt fetotoxic effects of the halogenated aromatic hydrocarbons are consistent with an Ah receptor-mediated mechanism. Nevertheless, one finding that stands out as being inconsistent is that 2,2′,3,3′,4,4′-HCB, which has a very weak if any affinity for binding to the Ah receptor, causes the same pattern of fetotoxic effects in mice as TCDD (Marks and Staples, 1980).

5.2.1.5. *Humans*

In the Yusho and Yu-Cheng poisoning episodes, developmental toxicity was reported in babies born to affected mothers who consumed rice oil contaminated with PCBs, CDFs, and PCQs (Hsu et al., 1985; Yamashita and Hayashi, 1985; Kuratsune, 1989; Rogan, 1989). In these incidents, it is essentially impossible to determine the contribution of TCDD-like versus non-TCDD-like congeners to the fetal/neonatal toxicity. Nevertheless, high perinatal

mortality was observed among hyperpigmented infants born to affected Yu-Cheng women who themselves did not experience increased mortality (Hsu et al., 1985). Thus, in humans the developing embryo/fetus may be more sensitive than the intoxicated mother to mortality caused by halogenated aromatic hydrocarbons.

In most cases, women who had affected children in the Yusho and Yu-Cheng episodes had chloracne themselves (Rogan, 1982). Based on this evidence, Rogan (1982) suggested that "exposure to amounts insufficient to produce some effect on the mother probably lessens the chance of fetopathy considerably." In support of this interpretation, overt signs of halogenated aromatic hydrocarbon toxicity were not observed in infants born to apparently unaffected mothers in the Seveso, Italy, and Times Beach, Missouri, TCDD incidents (Reggiani, 1989; Hoffman and Stehr-Green, 1989).

In laboratory mammals, the studies summarized previously in Table 5-1 have indicated an apparent association between prenatal mortality and maternal toxicity in nonprimate species. However, some TCDD-exposed rhesus monkeys were not able to carry their pregnancies to term even in the absence of any overt signs of maternal toxicity. This result in monkeys indicates that the relationship between maternal toxicity and any prenatal toxic effects on the human embryo/fetus must be cautiously defined. More data may be required to determine whether there is any association between overt maternal toxicity and embryo/fetal toxicity in both monkeys and humans.

Effects of chemical exposure on normal development of the human fetus can have four outcomes depending on the dose and time during gestation when exposure occurs: fetal death, growth retardation, structural malformations, and organ system dysfunction. In the Yusho and/or Yu-Cheng incidents, all of these outcomes were found (Yamashita and Hayashi, 1985; Kuratsune, 1989; Rogan, 1989). Increased prenatal mortality and low birthweight suggesting fetal growth retardation were observed in affected Yusho and Yu-Cheng women (Wong and Hwang, 1981; Law et al., 1981; Yamashita and Hayashi, 1985; Hsu et al., 1985; Miller, 1985; Lan et al., 1989; Rogan et al., 1988). A structural malformation, rocker bottom heel, was observed in Yusho infants (Yamashita and Hayashi,

1985). Organ dysfunction involving the CNS that was characterized by delays in attaining developmental milestones and by neurobehavioral abnormalities was reported in Yu-Cheng children exposed transplacentally (Rogan et al., 1988).

Organs and tissues that originate from embryonic ectoderm are well-known targets for toxicity following exposure to TCDD-like halogenated aromatic hydrocarbons. For example, treatment of adult monkeys with TCDD results in effects involving the skin, meibomian glands, and nails (Allen et al., 1977). Similarly, a hallmark sign of fetal/neonatal toxicity in the Yusho and Yu-Cheng episodes is an ectodermal dysplasia syndrome. It is characterized by hyperpigmentation of the skin and mucous membranes, hyperpigmentation and deformation of fingernails and toenails, hypersecretion of the meibomian glands, conjunctivitis, gingival hyperplasia, presence of erupted teeth in newborn infants, altered eruption of permanent teeth, missing permanent teeth, and abnormally shaped tooth roots (Taki et al., 1969; Yamaguchi et al., 1971; Funatsu et al., 1971; Wong and Hwang, 1981; Hsu et al., 1985; Yamashita and Hayashi, 1985; Rogan et al., 1988; Kuratsune, 1989; Rogan, 1989; Lan et al., 1989). Accelerated tooth eruption has been observed in newborn mice exposed to TCDD by lactation (Madhukar et al., 1984), as well as in the human infants mentioned above. In addition, other effects have been reported in Yusho and Yu-Cheng exposed infants that resemble those observed following TCDD exposure in adult monkeys. These include subcutaneous edema of the face and eyelids (Allen et al., 1977; Moore et al., 1979; Law et al., 1981; Yamashita and Hayashi, 1985; Rogan et al., 1988). Also, larger and wider fontanels and abnormal lung auscultation were found in the human infants (Law et al., 1981; Yamashita and Hayashi, 1985; Rogan et al., 1988). The similarities between certain effects reported in human infants exposed during the Yusho and Yu-Cheng incidents, as well as in adult monkeys and neonatal mice exposed to TCDD, enhance the probability that certain effects reported in human infants were caused by the TCDD-like PCB and CDF congeners in the contaminated rice oil ingested by the mothers of these infants.

Although chloracne is the most often cited effect of TCDD exposure involving the skin in adult humans, has an animal correlate in the hairless mouse, and can be studied by

using a mouse teratoma cell line in tissue culture (Poland and Knutson, 1982), it has rarely been recognized in the TCDD literature that the nervous system, like the skin, is derived from embryonic ectoderm (Balinsky, 1970). As will be described in Section 5.2.3.2, neurobehavioral effects occur following transplacental and neonatal exposure to TCDD-like congeners in mice, as well as transplacental exposure to TCDD itself in monkeys. In addition, in some of the Yu-Cheng children that were exposed transplacentally to PCBs, PCDFs, and PCQs there was a clinical impression of developmental delay or psychomotor delay including impairment of intellectual development (Rogan et al., 1988). As there is a clustering of effects due to TCDD-induced toxicity in organs derived from ectoderm, it is possible to speculate that direct effects of TCDD-like congeners on the central nervous system are responsible for some of the neurobehavioral effects observed in these children. Effects of TCDD on EGF receptors are associated with certain aspects of the ectodermal dysplasia syndrome such as hyperkeratinization of the skin (Osborne and Greenlee, 1985) and accelerated tooth eruption (Madhukar et al., 1984). Decreased autophosphorylation of the EGF receptor in human placentas is associated with decreased birthweight in infants born to exposed mothers 4 years after the initial Yu-Cheng exposure incident (Sunahara et al., 1987). This last result supports the earlier conclusion that careful study is needed to define the relationship between maternal toxicity, placental toxicity, and developmental toxicity in humans. In addition, further research is needed to characterize and elucidate the mechanisms by which TCDD affects the nervous system.

5.2.2. Structural Malformations

Developmental effects consisting of cleft palate, hydronephrosis, and thymic hypoplasia are produced in mice following in utero exposure to halogenated dibenzo-*p*-dioxin, dibenzofuran, biphenyl, and naphthalene congeners, which bind stereospecifically to the Ah receptor (Weber et al., 1985; Birnbaum et al., 1987a,b, 1991). Of these effects in the mouse, cleft palate is less responsive than hydronephrosis, as the latter is induced in the absence of cleft palate (Couture et al., 1990b). Both responses can be induced at TCDD

doses that are not otherwise overtly toxic (Couture et al., 1990a). The potency of TCDD for producing teratogenesis in the mouse is clearly evident when one considers that only 0.0003 percent of a maternally administered dose can be isolated from the fetal palatal shelves or kidneys. More specifically, a maternal TCDD dose of 30 μg/kg administered on gestational day 11 results in a tissue concentration of 0.65 pg TCDD/mg in the palatal shelves 3 days after dosing, and the same tissue concentration of TCDD is present in the kidneys at that time (Abbott et al., 1989).

Susceptibility to the developmental actions of TCDD in mice depends on two factors: genotype of the fetus and stage of development at the time of exposure. The Ah receptor is thought to mediate the developmental effects of TCDD (Poland and Knutson, 1982). Mouse strains that produce Ah receptors with relatively high affinity for TCDD respond to lower doses of TCDD than mouse strains that produce relatively low-affinity Ah receptors (Poland and Glover, 1980; Hassoun et al., 1984a). Thus, one genetically encoded parameter that determines the responsiveness of different mouse strains is the Ah receptor protein itself.

The differences that exist between mouse strains with respect to developmental responsiveness to these chemicals are not absolute, as all strains, including those with Ah receptors of relatively low affinity, respond when exposed to sufficiently large doses during the critical period of organogenesis (Birnbaum, 1991). In the mouse, the peak times of fetal sensitivity vary slightly depending on which developmental effect is used as the endpoint. However, exposure between days 6 and 15 of gestation will produce teratogenesis (Couture et al., 1990a,b).

In inbred strains of mice, the developmental response, characterized by altered cellular proliferation, metaplasia, and modified terminal differentiation of epithelial tissues (Poland and Knutson, 1982), is extremely organ specific, occurring only in the palate, kidney, and thymus (Birnbaum, 1991). Pharmacokinetic differences are not responsible for this high degree of tissue specificity, and Ah receptors are not found exclusively in the affected organs (Carlstedt-Duke, 1979; Gasiewicz et al., 1983). Therefore, other factors intrinsic to the palate, kidney, and thymus appear to play a role along with the Ah receptors

in these tissues in producing the structural malformations. For certain developmental effects, the time at which exposure occurs is important, as there may be a critical period during which the toxicant must be present in order to produce the effect. This critical period can be different for different organs and tissues.

Differences exist between mammalian species with respect to susceptibility to the developmental effects of TCDD. Although genetic differences between species or strains might affect absorption, biotransformation, and/or elimination of TCDD by the maternal system and its absorption across the placenta, such species differences do not account for the lack of cleft palate formation in species other than mice (Birnbaum, 1991). Rather, the species differences in susceptibility to cleft palate formation appear due to differences in the interaction between TCDD and the developing palatal shelves themselves. This is demonstrated by the occurrence of similar responses when palatal shelves from different species are exposed to TCDD in organ culture (Abbott et al., 1989; Abbott and Birnbaum, 1990a, 1991). The key difference is that much higher concentrations of TCDD are required to elicit essentially the same palatal response that is seen in the mouse in other species (Table 5-3).

With respect to the occurrence of similar developmental effects in mammalian species other than the mouse, no other species develop cleft palate except at maternal doses that are fetotoxic and maternally toxic (Couture et al., 1990a; Birnbaum, 1991). In mice and hamsters, hydronephrosis can be elicited at TCDD doses that are neither fetotoxic nor maternally toxic (Olson and McGarrigle, 1991), whereas thymic hypoplasia is a fetal response to TCDD observed in virtually all laboratory mammalian species that have been tested (Vos and Moore, 1974). Studies in humans have not clearly identified an association between TCDD exposure and structural malformations (Fara and Del Corno, 1985; Mastroiacovo et al., 1988; Stockbauer et al., 1988; Reggiani, 1989).

TABLE 5-3
TCDD Responsiveness of Palatal Shelves From the Mouse, Rat, and Human in Organ Culture

Species	Molar concentration of TCDD		
	Induction of Epithelial Proliferation and Prevention of Epithelial-To-Mesenchyme Transformation		Cytotoxicity
	LOEL	EC_{100}	
Mouse	$1x10^{-13}$	$5x10^{-11}$	$1x10^{-10}$
Rat[a]	$1x10^{-10}$	$1x10^{-8}$	$1x10^{-7}$
Human[b]	$5x10^{-11}$	$1x10^{-8}$	$1x10^{-7}$

[a]At the highest concentration tested, 60 percent of the palatal shelves failed to undergo programmed cell death.
[b]One of four shelves responded by failing to undergo programmed cell death at $5x10^{-11}$ M.

Source: Birnbaum, 1991.

5.2.2.1. *Cleft Palate*

5.2.2.1.1. ***Characterization of TCDD effect.*** Palatal shelves in the mouse originate as outgrowths of the maxillary process. Eventually, they come to lie vertically within the oral cavity on both sides of the tongue. In order to form the barrier between the oral and nasal cavities, the shelves in the mouse must reorient themselves from a vertical direction to a horizontal direction. Once they come together horizontally, their medial aspects bring apposing epithelia into close contact (Coleman, 1965; Greene and Pratt, 1976). At this stage, the apposing medial edge epithelia of the separate palatal shelves each consist of an outer layer of periderm that overlays a strata of cuboidal-shaped basal cells. These basal cells, in turn, rest on top of a continuous basal lamina. There is a sloughing of the outer periderm cells followed by the formation of junctions between the newly apposing basal epithelial cells. The midline seam so formed consists of the two layers of basal cells, all of which appear healthy, even though the outer periderm cells are shed before adhesion occurs. As fusion proceeds, the bilayer seam breaks up into small islands of cells. Eventually, the basal lamina disappears and the elongating former basal cells within the small islands extend filopodia into the adjacent connective tissue. During this process, the former basal cells lose epithelial characteristics and gain fibroblast-like features. Essentially, the medial edge epithelium is an ectoderm that retains the ability to transform into mesenchymal cells. Upon completion of this epithelial to mesenchyme transformation, the once separate and apposing palatal shelves are fused so that a single continuous tissue is formed (Fitchett and Hay, 1989; Shuler et al., 1991).

Cleft palate can result from a failure of the shelves to grow and come together or a failure of the shelves to fuse once they are in close apposition (Pratt et al., 1985). TCDD and other Ah receptor agonists are unusual inducers of cleft palate because the shelves grow and make contact, but the subsequent processes involving loss of periderm, shelf adhesion, and the epithelial to mesenchyme transformation does not occur. Therefore, a cleft is formed as the palatal shelves continue to grow without fusing. When TCDD is administered to pregnant mice on gestational days 6 to 12, the incidence of cleft palate formation increases

with time. However, day 12 is a critical window, after which the incidence of cleft palate formation decreases. No cleft palates are formed when TCDD is administered on day 14 (Couture et al., 1990b).

Palatal shelves of the mouse, rat, and human can be removed from the fetus and placed into organ culture. Under these conditions, when the separate shelves are placed in an apposing condition in vitro, sloughing periderm cells are trapped within the seam (Fitchett and Hay, 1989). Thus, due to the presence of these trapped dead cells, the fusion process was previously believed to require programmed cell death to remove epithelial cells at the fusion seam (Coleman, 1965; Greene and Pratt, 1976; Pratt et al., 1984). However, the newer model, which involves transformation of the basal epithelial cells into mesenchyme rather than their death, is believed to be valid under explant conditions in vitro, as well as in vivo (Fitchett and Hay, 1989). When exposed to TCDD as explants in vitro, the palatal shelves of the mouse, rat, and human all respond to TCDD in a similar way by retaining medial epithelial cells that proliferate and differentiate into a stratified epithelium (Abbott et al., 1989; Abbott and Birnbaum, 1989, 1990a, 1991). The epithelial to mesenchyme transformation of the basal epithelial cells does not occur, and instead there is a differentiation into a stratified squamous epithelium such that these cells resemble the squamous keratinizing oral cells within the tissue (Birnbaum and Abbott, personal communication).

Table 5-3 shows the lowest TCDD concentration that prevents the epithelial to mesenchyme transformation process in isolated palatal shelves (LOEL), TCDD concentration that produces a 100 percent maximal response (EC_{100}), and lowest concentration of TCDD that produces cytotoxicity. Palatal shelves of rats and humans respond to TCDD in a manner identical to the mouse; however, higher concentrations of TCDD are required to induce the epithelial responses. The relative insensitivity of rat palatal shelves may explain the lack of cleft palate when fetal rats are exposed to nonmaternally toxic doses of TCDD. Sensitivity of human palatal shelves to TCDD in vitro is similar to the rat. This suggests that exposure

to maternally toxic and fetotoxic doses of TCDD would be required to cause cleft palate formation in humans.

A disruption in the normal spatial and temporal expression of EGF, TGF-α, TGF-β1, and TGF-β2 correlates with altered proliferation and differentiation in the medial region of the developing palate, resulting in a palatal cleft. Thus, the abnormal proliferation and differentiation of TCDD-exposed medial cells may be related to reduced expression of EGF and TGF-α. Also, decreased levels of immunohistochemically detectable TGF-β1 could contribute to the continued proliferation and altered differentiation of medial cells (Abbott and Birnbaum, 1990b). It is important to note that EGF and TGF-α both exert their actions by binding to EGF receptors. The differentiation of basal cells to a stratified squamous epithelium, which resembles the keratinizing oral epithelium within the developing palate that is mentioned above, is similar to certain effects of TCDD that can be studied in cultured human keratinocytes. These effects in cultured human keratinocytes involve altered EGF binding to those cells. In addition, the Ah receptor is implicated in producing this response in cultured cells (Osborne and Greenlee, 1985). Thus, the mechanisms by which TCDD produces a palatal cleft in the mouse may have similarities to the mechanisms by which TCDD produces other effects that are part of the ectodermal dysplasia syndrome. This is consistent with the description given by Fitchett and Hay (1989) that the medial edge epithelium within the developing palate is essentially an ectoderm that retains the ability to transform into mesenchymal cells.

5.2.2.1.2. ***Evidence for an Ah receptor mechanism.***

5.2.2.1.2.1. ***Genetic.*** When wild-type C57BL/6 (Ah^bAh^b) mice are crossed with DBA/2 (Ah^dAh^d) mice that contain a mutation at the Ah locus, all of the heterozygous B6D2F1 progeny (Ah^bAh^d) resemble the wild-type parent in that AHH activity is inducible by TCDD and other halogenated aromatic hydrocarbons (Nebert and Gielen, 1972). Test crosses between the B6D2F1 progeny and each original parent strain, and other B6D2F1 progeny mice, demonstrate that in the C57BL/6 and DBA/2 strains, susceptibility to AHH induction

segregates as a simple dominant trait in the backcross and F_2 progeny. Thus, the trait of AHH induction is expressed in progeny that contain the Ah^bAh^b and Ah^bAh^d genotypes, but is not expressed in the Ah^dAh^d progeny from these crosses. Certain other effects of TCDD, such as its binding affinity for the hepatic Ah receptor (Okey et al., 1979), thymic atrophy (Poland and Glover, 1980), hepatic porphyria (Jones and Sweeney, 1980), and immunosuppressive effects (Vecchi et al., 1983; Nagarkatti et al., 1984) have been shown in similar genetic crosses and test crosses to segregate with the Ah locus that permits AHH induction. Thus, for these effects of TCDD, genetic evidence demonstrates an involvement of the Ah locus (Poland and Knutson, 1982).

Nebert's group was the first to relate developmental toxicity to the Ah locus in mice (Lambert and Nebert, 1977; Shum et al., 1979). Subsequently, Poland and Glover (1980) administered a single 30 μg TCDD/kg dose to pregnant mice on gestational day 10. It was found that there was a 54 percent incidence of cleft palate in homozygous C57BL/6 (Ah^bAh^b) fetuses, a 13 percent incidence in heterozygous B6D2F1 (C57BL/6 and DBA/2 hybrid, Ah^bAh^d) fetuses, and only a 2 percent incidence in homozygous DBA/2 (Ah^dAh^d) fetuses. This pattern of inheritance, in which the incidence of developmental toxicity in the heterozygous F1 generation is intermediate between that of the homozygous parental strains, is consistent with the autosomal dominant pattern of inheritance described for AHH inducibility and the Ah locus (Nebert and Gielen, 1972), even if dominance is incomplete in the case of developmental toxicity. However, the pattern of inheritance for developmental toxicity described when Poland and Glover (1980) crossed C57BL/6 and DBA/2 mice is not sufficient proof that the Ah locus is the genetic locus that controls susceptibility to TCDD-induced developmental toxicity in these mouse strains.

To provide such proof, it is necessary to show genetic linkage between the susceptibility for developmental toxicity and the Ah locus. The standard of proof would be that developmental toxicity and a particular allele at the Ah locus must always segregate together in genetic crosses because if the loci are the same there can be no recombination between the loci. This is generally accomplished by demonstrating cosegregation between

the two loci, not only in crosses between the two homozygous parental strains, which in and of itself is insufficient proof of genetic linkage, but also in test crosses or backcrosses between the heterozygous F1 hybrids with each homozygous parental strain.

It was stated previously that certain effects of TCDD are well known to segregate with the Ah locus due to the results of appropriate crosses and backcrosses between responsive and nonresponsive mouse strains and their hybrid F1 progeny. With this standard of proof in mind, the evidence that specifically links developmental toxicity with the Ah locus can be described. It is intended that this information be provided with a considerable degree of detail, so the reader can independently determine whether the standard of proof has been satisfied by the evidence available.

To strengthen their conclusion based on the results of simple crosses between C57BL/6 and DBA/2 mice, Poland and Glover (1980) planned to perform a backcross between the hybrid B6D2F1 and DBA/2. However, the low incidence of cleft palate in B6D2F1 mice would have required characterizing and phenotyping a prohibitively large number of fetuses. Alternatively, the backcross between B6D2F1 and C57BL/6 was considered, in which Ah^bAh^b and Ah^bAh^d progeny would have been distinguished by the amount of high-affinity specific binding for TCDD in fetal liver. In this case, however, overlap between individual mice would have made the results uncertain in some of the progeny. Therefore, it was not possible to obtain satisfactory results from either backcross.

Instead, Poland and Glover (1980) examined the incidence of cleft palate in 10 inbred strains of mice: 5 strains with high-affinity Ah receptors and 5 strains with low-affinity receptors. In the five latter strains, there was only a 0 to 3 percent incidence of cleft palate formation, whereas four of the five strains with high-affinity Ah receptors developed a ≥ 50 percent incidence. The one strain with high-affinity Ah receptors that did not follow the pattern, CBA strain, is also resistant to cleft palate formation induced by glucocorticoids. Overall, these results indicate that cleft palate formation probably segregates with the Ah locus.

The incidence of cleft palate formation was studied in fetuses from a cross between C57BL/6 and AKR/NBom mice administered 3,3′,4,4′-TCAOB on gestational day 12 (Hassoun et al., 1984b). Although C57BL/6 mice are responsive for AHH induction and cleft palate formation, AKR mice are less responsive, requiring higher doses for both effects. In a manner unlike the result of a cross between C57BL/6 and DBA/2, the incidence of cleft palate formation in the B6AKF1 progeny was <2 percent, showing that nonresponsiveness segregates as the dominant trait when C57BL/6 mice are crossed with AKR mice. Similarly, cleft palate formation was virtually absent in the progeny of a backcross between AKR/NBom and B6AKF1, demonstrating dominance of the noninducible trait. Although Ah phenotyping of the backcross progeny was not performed in this particular study, Robinson et al. (1974) had previously evaluated segregation of the Ah locus in backcrosses between C57BL/6 and AKR/N mice. They found in these two strains that noninducibility for AHH activity segregates as the dominant trait. Thus, inducibility for cleft palate formation and AHH activity both segregate as dominant traits when C57BL/6 mice are crossed with DBA/2, but noninducibility is dominant for both traits when C57BL/6 mice are crossed with AKR/N. These results are consistent with the interpretation that cleft palate induction probably segregates with the Ah locus.

Like Poland and Glover (1980), Hassoun et al. (1984a) were unable to determine whether cleft palate formation segregates with the Ah locus in C57BL/6 and DBA/2 mice by performing simple backcrosses. Instead, they evaluated cosegregation of the Ah locus and 2,3,7,8-TCDF-induced cleft palate formation using a series of recombinant strains called BXD mice. These strains are fixed recombinants produced from an original cross between the two parental strains C57BL/6J and DBA/2J. Hybrid B6D2F1 mice were crossed to produce F_2 progeny and these were strictly inbred by sister and brother matings into several parallel strains. The mice used in this study were from the F_{42} and F_{58} generations of inbreeding. It was found that the incidence of TCDF-induced cleft palate formation after matings within eight different BXD strains with high-affinity Ah receptors is >85 percent. After similar matings with eight different BXD strains with low-affinity Ah receptors, the

incidence of TCDF-induced cleft palate formation is <2 percent. These results of Hassoun et al. (1984a) corroborate those of Poland and Glover (1980) and provide the best evidence currently available that cleft palate formation segregates with the Ah locus. Thus, the Ah locus and the Ah receptor are involved in the formation of palatal clefts that are induced by TCDD-like congeners.

As additional evidence, stereospecific, high-affinity Ah receptors can be isolated from cytosol fractions prepared from embryonic palatal shelves. These receptors are present in palatal shelves of Ah^bAh^b, C57BL/6 fetuses but are not detectable in similar tissue from Ah^dAh^d, AKR/J fetuses (Dencker and Pratt, 1981). However, the significance of this finding may be mitigated to some extent by the following observation. In cytosols prepared from homogenates of whole embryo/fetal tissue (minus head, limbs, tail, and viscera), the concentration of specific binding TCDD receptors is 256 fmol/mg protein in C57BL/6 mice, compared with a concentration of 21 fmol/mg protein in the less responsive DBA/2 strain, 15 fmol/mg protein in the less responsive AKR/J strain, and 19 fmol/mg protein in the less responsive SWR/J strain. However, when embryonic tissue is cultured, the differences between the strains in receptor number are less pronounced, and in the receptors isolated from cultured embryonic cells of different strains, there is only about a twofold difference in the relative binding affinity for ^{3}H-TCDD. The mechanistic reasons for the diminished degree of difference between responsive and less responsive mouse strains during embryonic cell culture are not known (Harper et al., 1991).

The possible influence of maternal toxicity on cleft palate formation was evaluated by performing reciprocal blastocyst transfer experiments using the high-affinity-Ah receptor NMRI and lower affinity-Ah receptor DBA strains of mice (D'Argy et al., 1984). After administration of 30-μg TCDD/kg or 8 mg TCAOB/kg to pregnant dams on gestational day 12, 75 to 100 percent of all NMRI fetuses developed cleft palates. This is true whether the fetuses remained within the uterus of their natural mother or were transferred into the uterus of a DBA mouse. Under the same conditions, none of the 24 DBA fetuses transferred into an NMRI mother developed a cleft palate, even though 89 percent of their NMRI litter mates

were affected. Thus, these results, along with the presence of Ah receptors in palatal shelves and responsiveness of palatal shelves in organ culture to TCDD, indicate that cleft palate formation in mice is due to a direct effect of TCDD on the palatal shelf itself and is not secondary to maternal toxicity.

5.2.2.1.2.2. ***Structure activity.*** As genetic evidence in mice indicates that the Ah receptor mediates TCDD-induced cleft palate formation and hydronephrosis (see Section 5.3.2.2.2.1), structure-activity requirements based on Ah receptor-binding characteristics should predict the relative potencies of different agonists for producing cleft palate and hydronephrosis. Of the halogenated aromatic hydrocarbons, TCDD has the greatest affinity for binding to the Ah receptor and it is the most potent teratogen in inbred mouse strains. Table 5-4 shows the relative potencies for cleft palate induction and hydronephrosis in C57BL/6 mice for a number of TCDD-like congeners. As TCDD is the most potent, it is assigned a value of 1.000. When examined by probit analysis, the dose response curve of each congener, compared with all of the others, did not deviate from parallelism. Therefore, the relative potencies of the congeners are valid for any given incidence of cleft palate formation or hydronephrosis. The main finding, however, is that the rank order potency of the various congeners for producing these two developmental effects is generally similar to that for binding to the Ah receptor (see Table 5-4), with the notable exception that the apparent binding affinities for the brominated dibenzofurans have not yet been reported. There are additional ligands for the Ah receptor that cause cleft palate formation in C57BL/6 mice at nonmaternally toxic doses, but they are not listed in the table. These include 3,3′,4,4′-TCAOB (Hassoun et al., 1984a), 3,3′,4,4′-tetrachlorobiphenyl (Marks et al., 1989), 3,3′,4,4′,5,5′-hexachlorobiphenyl (Marks et al., 1981), and a mixture that contained 1,2,3,4,6,7- and 2,3,4,5,6,7-hexabromonaphthalenes (Miller and Birnbaum, 1986).

Also consistent with the structure-activity relationships for binding to the Ah receptor is the finding that a number of hexachlorobiphenyls do not induce cleft palate formation. These congeners either lack sufficient lateral substitution or are substituted in such a manner

TABLE 5-4
Relative Teratogenic Potency of Halogenated Aromatic Hydrocarbon Congeners in C57BL/6 Mice

Congener	Relative potency (ED_{50} TCDD/ED_{50} congener)	
	Cleft palate	Hydronephrosis
2,3,7,8-TCDD	1.000	1.000
2,3,7,8-TBDD	0.235	0.444
2,3,7,8-TBDF	0.100	0.333
2,3,4,7,8-PeCDF	0.095	0.057
2,3,7,8-TCDF	0.049	0.021
1,2,3,7,8-PeCDF	0.026	0.074
1,2,3,4,7,8-HxCDF	0.010	0.049
2,3,4,7,8-PeBDF	0.005	0.009
1,2,3,7,8-PeBDF	0.004	0.018
2,3,4,5,3',4'-HxCB	0.0000287	0.0000894

Source: Weber et al., 1985; Birnbaum et al., 1987a,b, 1991.

that they cannot achieve a planar conformation. Included in this category are the diortho and tetraortho chlorine-substituted 2,2′,3,3′,5,5′-, 2,2′,3,3′,6,6′-, 2,2′,4,4′5,5′-, and 2,2′,4,4′,6,6′-hexachlorobiphenyls (Marks and Staples, 1980). In addition, it is consistent with the structure-activity relationships that the monoortho chlorine-substituted 2,3,4,5,3′,4′-HCB is a weak teratogen. Its potency relative to that of TCDD varies from $3x10^{-5}$ to $9x10^{-5}$ for cleft palate formation, AHH induction, and hydronephrosis (see Table 5-4) (Kannan et al., 1988).

A result that would not be expected according to the structure-activity relationships for binding to the Ah receptor is that the diortho chlorine-substituted 2,2′,3,3′,4,4′-hexachlorobiphenyl causes cleft palate formation and hydronephrosis in mice (Marks and Staples, 1980). However, another diortho chlorine-substituted PCB congener, 2,2′,4,4′,5,5′-hexachlorobiphenyl, also can cause hydronephrosis and is a very weak inducer of EROD activity (Biegel et al., 1989; Morrissey et al., 1992). It is consistent with the interpretation that 2,2′,4,4′,5,5′-hexachlorobiphenyl is a partial Ah receptor agonist that it can competitively displace TCDD from the murine hepatic cytosolic receptor and, at large enough doses, can inhibit TCDD-induced cleft palate formation and immunotoxicity in C57BL/6 mice (Biegel et al., 1989; Morrissey et al., 1992). These results suggest that PCB congeners do not have to be in a strictly planar configuration to cause teratogenesis.

5.2.2.1.3. ***Species differences.*** Cleft palate is induced in rats only at maternally toxic TCDD doses that are associated with a high incidence of fetal lethality. Schwetz et al. (1973) reported an increased incidence of cleft palate after maternal administration of 100 μg hexachlorodibenzo-*p*-dioxin/kg/day to Sprague-Dawley rats on days 6 to 15 of gestation. Couture et al. (1989) also observed an increased incidence of cleft palate formation after a single dose of 300 μg/kg of 2,3,4,7,8-pentachlorodibenzofuran given to Fischer 344 rats. Similarly, cleft palate can be produced in fetal hamsters following maternally toxic and fetotoxic doses of TCDD (Olson et al., 1990).

In monkeys, bifid uvula (Zingeser, 1979) and bony defects in the hard palate (McNulty, 1985) were reported, but there were no corresponding soft tissue defects or clefts of the secondary palate. Cleft palates have not been reported in human fetuses of mothers accidentally exposed to TCDD or mixtures of PCBs and CDFs (Fara and Del Corno, 1985; Mastroiacovo et al., 1988; Stockbauer et al., 1988; Rogan, 1989). Thus, sensitivity of the palate in mice to TCDD is unique. In other species, including humans, other forms of fetal toxicity occur at doses lower than those required for cleft palate formation.

5.2.2.2. *Hydronephrosis*

5.2.2.2.1. ***Characterization of TCDD effect.*** Hydronephrosis is the most sensitive developmental response elicited by TCDD in mice. It is produced by maternal doses of TCDD too low to cause palatal clefting and is characterized as a progressive hydronephrosis preferentially occurring in the right kidney, which can be accompanied by hydroureter and/or abnormal nephron development (Courtney and Moore, 1971; Moore et al., 1973; Birnbaum et al., 1985; Weber et al., 1985; Abbott et al., 1987a; 1987b). Hyperplasia of the ureteric lumenal epithelium results in ureteric obstruction. Therefore, the TCDD-induced kidney malformation in the mouse is a true hydronephrosis in that blockage of urine flow results in back pressure damaging or destroying the renal papilla (Abbott et al., 1987a).

When dissected on gestational day 12 from control embryos, isolated ureters exposed to $1x10^{-10}$ M TCDD in vitro display evidence of epithelial cell hyperplasia (Abbott and Birnbaum, 1990c). This is significant in that it shows that the hydronephrosis response is due to a direct effect of TCDD on the ureteric epithelium. Embryonic cell proliferation within the ureter may be regulated by the actions of growth factors, including EGF (Abbott and Birnbaum, 1990c). In control ureteric epithelia, the expression of EGF receptors decreases with advancing development, whereas after TCDD exposure the rate of ^{3}H-thymidine incorporation and expression of EGF receptor does not decline. Therefore, in TCDD-treated mice there is a correlation between excessive proliferation of ureteric epithelial cells and inappropriate expression of EGF receptors.

Other effects of TCDD on the developing kidney involve changes in the extracellular matrix components and basal lamina (Abbott et al., 1987b). In TCDD-exposed fetal kidneys, extracellular matrix fibers are of a diameter consistent with Type III collagen similar to such fibers in unexposed fetal kidneys. However, the abundance of these Type III collagen fibers is reduced by TCDD treatment. In the developing kidney, these collagen fibers are associated with undifferentiated mesenchymal cells. Similarly, the expression of fibronectin, which is also associated with undifferentiated mesenchymal cells, is decreased by TCDD exposure. In the glomerular basement membrane, the distribution of laminin and Type IV collagen is altered by TCDD exposure. These changes in the glomerular basement membrane may affect the functional integrity of the filtration barrier and could exacerbate the hydronephrosis and hydroureter. The proteins within the extracellular matrix and basal lamina that are altered by TCDD exposure—laminin, fibronectin, and collagen—are considered markers of a commitment to differentiate into epithelial structures. In the mouse embryo/fetus, TCDD exposure also blocks differentiation within the epithelium of the developing palate. Although there are effects of TCDD exposure on EGF in the developing ureter as well as the developing palate, the urinary system, unlike parts of the soft palate, is derived from mesoderm. Thus, it is important to note that the ectodermal-dysplasia syndrome is intended to denote a clustering of effects that appears to involve ectoderm-derived organs. It is not intended to imply that all TCDD-induced developmental toxicity involves organs derived from ectoderm.

5.2.2.2.2. ***Evidence for an Ah receptor mechanism.***

5.2.2.2.2.1. ***Genetic.*** With respect to involvement of the Ah locus in TCDD-induced hydronephrosis, very few genetic studies have been done. Prior to the discovery of the Ah locus, however, Courtney and Moore (1971) reported a 62 percent incidence of hydronephrosis in C57BL/6 mice exposed to a maternal TCDD dose of 3 μg/kg/day on days 6 to 15 of gestation, whereas the incidence in similarly exposed DBA/2 mice was only 26 percent. More recently, Silkworth et al. (1989) reported that when TCDD is administered

on gestational days 6 to 15, the incidence of hydronephrosis is dose related. As the maternal dose of TCDD is increased from 0.5 to 4 μg/kg/day, the incidence of hydronephrosis in C57BL/6 mice increases from 31 to 92 percent, whereas in DBA/2 mice the incidence varies from 5 to 37 percent over the same dose range. In DBA/2 mice the incidence of hydronephrosis increases to 60 percent when the largest dose of TCDD administered is doubled to 8 μg/kg/day (but does not reach the 92 percent level seen in C57BL/6 mice at 4 μg TCDD/kg). Thus, the incidence of hydronephrosis is higher in the mouse strain that produces high-affinity Ah receptors (C57BL/6) compared with that strain (DBA/2) that produces Ah receptors having lower ligand-binding affinity (Okey et al., 1989). The largest dose of TCDD used in these experiments resulted in hydronephrosis of the fetus without affecting the mean body weight or body weight gain of the dam. In the BXD strains (Hassoun et al., 1984a), the incidence of 2,3,7,8-TCDF-induced hydronephrosis is 34 to 48 percent in eight strains with high-affinity Ah receptors and 3 to 4 percent in eight strains with low-affinity Ah receptors. These results obtained in the BXD strains of mice provide the best evidence currently available of an association between the ability of TCDD-like congeners to induce hydronephrosis and the wild-type Ah^b allele. Thus, the Ah locus and the Ah receptor are involved in the hydronephrosis that is induced by TCDD-like congeners.

5.2.2.2.2.2. ***Structure activity.*** The rank order of potencies for various halogenated aromatic hydrocarbon congeners to cause hydronephrosis in mice is consistent with the structure-activity requirements for binding to the Ah receptor (see Table 5-4). This provides further evidence that the Ah receptor mediates the effects of these TCDD-like congeners on the developing mouse kidney.

5.2.2.2.3. ***Species differences.*** Hydronephrosis has been reported after administration of low maternal doses of TCDD to rats and hamsters. Possibly due to the small numbers of fetuses examined, the observed incidences of hydronephrosis in rats after exposure to cumulative maternal doses <2 μg TCDD/kg have not been statistically significant (Courtney

and Moore, 1971; Giavini et al., 1983). On the other hand, following a 1.5 μg TCDD/kg dose administered on gestational days 7 and 9, the incidence of hydronephrosis in hamster fetuses was 11 and 4.2 percent, respectively. This is in contrast to an incidence of < 1 percent in control hamster fetuses. Accordingly, hydronephrosis is one of the most sensitive indicators of prenatal toxicity in hamsters (Olson and McGarrigle, 1991).

5.2.3. Postnatal Effects

5.2.3.1. *Male Reproductive System*

TCDD has been shown to decrease plasma androgen concentrations in the adult male rat (see 5.3.2.2). Because TCDD is known to be transferred from mother to young in utero and during lactation (Moore et al., 1976; Van den Berg et al., 1987), it can be expected to have an impact on the male reproductive system during early development (Mably et al., 1991). Testosterone and/or its metabolite DHT are essential prenatally and/or early postnatally for imprinting and development of accessory sex organs (Chung and Raymond, 1976; Rajfer and Coffey, 1979; Coffey, 1988) and for initiation of spermatogenesis (Steinberger and Steinberger, 1989). In addition, aromatization of testosterone to 17ß-estradiol within the CNS is required perinatally for the imprinting of typical adult male patterns of reproductive behavior (Gorski, 1974) and LH secretion (Barraclough, 1980). Thus, normal development of male reproductive organs and imprinting of typical adult sexual behavior patterns require sufficient testosterone to be secreted by the fetal and neonatal testis at critical times in early development before and shortly after birth (MacLusky and Naftolin, 1981; Wilson et al., 1981). If perinatal imprinting has failed to occur in the accessory sex organs of a neonatal male rat, the result could be that these organs will not develop a normal trophic response to androgenic stimulation and will not grow in a normal way as the animal becomes sexually mature.

5.2.3.1.1. ***Overt toxicity assessment.*** To determine how the male reproductive system is affected by in utero and lactational TCDD exposure, Mably et al. (1991, 1992a,b,c) treated

pregnant rats with a single oral dose of TCDD (0.064, 0.16, 0.4, or 1.0 μg/kg) or vehicle on day 15 of gestation (day 0 = sperm positive). Day 15 was chosen because most organogenesis in the fetus is complete by this time and the hypothalamic/pituitary/testis axis is just beginning to function (Warren et al., 1975, 1984; Aubert et al., 1985). The pups were weaned 21 days after birth. The consequences of this single, maternal TCDD exposure for the male offspring were characterized at various stages of postnatal sexual development.

Mably et al. (1992a) found that TCDD treatment had no effect on daily feed intake during pregnancy and the first 10 days after delivery, nor did it have an effect on the body weight of dams on day 20 of gestation or on days 1, 7, 14, or 21 postpartum. Treating dams with graded doses of TCDD on day 15 of gestation had no effect on gestation index, length of gestation, or litter size. Except for an 8 percent decrease at the highest maternal dose, TCDD had no effect on live birth index. Neither the 4-day nor 21-day survival index was significantly affected by TCDD. In all dosage groups, the number of dead offspring was equally distributed between males and females and of the females that failed to deliver litters, none were pregnant. Signs of overt toxicity among the offspring were limited to the above mentioned 8 percent decrease in live birth index (highest dose only), initial 10 to 15 percent decreases in body weight (two highest doses), and initial 10 to 20 percent decreases in feed intake (measured for males only, two highest doses). The latter two effects disappeared by early adulthood, after which the body weights of the maternally exposed and nonexposed rats were similar. Gray et al. (1993) reported similar findings for offspring survival and growth following a 1 ug/kg TCDD exposure on day 15 of gestation in the Long Evans Hooded rat. No male offspring with gross external malformations were reported in either of these studies.

5.2.3.1.2. ***Androgenic status.*** The androgenic status of the male offspring can be determined from the structure and function of androgen-dependent systems and from the levels of circulating androgens. Mably et al. (1992a) examined the dose-related effect of TCDD exposure on several androgen-dependent endpoints. Anogenital distance, which is dependent on both circulating androgen concentrations and androgenic responsiveness

(Neumann et al., 1970), was reduced in 1- and 4-day-old male pups by a single maternal TCDD dose as low as 0.16 ug/kg, even when slight decreases in body length were considered. Gray et al. (1993) reported a similar reduction in male anogenital distance in the Long Evans rat, although the reduction was smaller and it appeared to be related to a decrease in body weight.

Endpoints of sexual maturation were also affected by TCDD exposure. Testis descent, an androgen-mediated developmental event that normally occurs in rats between 20 and 25 days of age (Rajfer and Walsh, 1977) was delayed up to 1.7 days by a single 0.16 ug/kg dose or above (Mably et al., 1992). At 1 ug/kg, preputial separation was delayed for 3 days (Gray et al., 1993). This latter endpoint was not evaluated at lower exposure levels.

Effects into adulthood were also observed. A single maternal exposure to TCDD on gestation day 15 led to a decrease in juvenile and adult ventral prostate weight and seminal vesicle weight in the offspring (Mably et al., 1992a; Gray et al., 1993). Mably et al. (1992a) examined a variety of androgen-dependent parameters of male offspring from postnatal day 32 to 120. The lowest single maternal TCDD dose employed (0.064 ug/kg) led to a significantly depressed ventral prostate weight at 32 days of age. There were trends (though not statistically significant) for plasma testosterone and DHT concentrations to be decreased at these times, although plasma LH concentrations were generally unaffected. An exception was a 95-percent decrease in plasma LH concentration on postnatal day 32 caused by a maternal TCDD dose of 1.0 μg/kg. The lowest maternal TCDD dose to affect a parameter of androgenic status was the lowest dose tested—0.064 μg/kg. This dose resulted in a significantly depressed ventral prostate weight at 32 days of age. When ventral prostate weight was indexed to body weight, however, 0.16 μg TCDD/kg was the lowest dose that caused a significant reduction in relative ventral prostate weight. Although there was no effect on the body weight of male pups at this dose, 0.16 μg TCDD/kg caused a consistent pattern of effects indicating a depression of androgenic status. The reductions in seminal vesicle and ventral prostate weights may be due to modest reductions in plasma androgen concentrations and/or androgen responsiveness caused by incomplete perinatal imprinting of

the accessory sex organs (Mably et al., 1992a). Table 5-5 summarizes these effects (Mably et al., 1991, 1992a).

To determine if in utero and lactational exposure to TCDD produces a perinatal androgenic deficiency, Mably et al. (1991, 1992a) dosed pregnant rats with 1.0 μg TCDD/kg on day 15 of gestation. Plasma testosterone concentrations were greater in control male than in control female fetuses on days 17 to 21 of gestation, particularly during the prenatal testosterone surge (days 17 to 19). On days 18 to 21 of gestation, TCDD exposure reduced the magnitude of this sex-based difference. Postnatally, plasma testosterone concentrations peaked 2 hours after birth in control males, whereas in TCDD-exposed males, the peak did not occur until 4 hours after birth and was only half as large. Contrasting these findings, recent findings reported in abstract form have not confirmed an effect of TCDD on androgen production. Neither Chen et al. (1993), repeating the exact Mably protocol (Mably et al., 1992a), nor Gray et al. (1993), using an identical TCDD exposure in a different rat strain, have demonstrated a significant decrease in plasma or testicular testosterone following TCDD exposure identical to that of Mably et al. (1992a).

5.2.3.1.3. ***Spermatogenesis.*** Mably et al. (1991, 1992c) found that decreased spermatogenesis was among the most sensitive responses of the male rat reproductive system to perinatal TCDD exposure. Testis and epididymis weights and indices of spermatogenesis were determined on postnatal days 32, 49, 63, and 120. Perinatal TCDD exposure caused dose-related decreases in testis and epididymis weights. Weights of the caudal portion of the epididymis where mature sperm are stored prior to ejaculation were decreased the most, by ~45 percent. The number of sperm per cauda epididymis was decreased by 75 and 65 percent on days 63 and 120, respectively, and appeared to be the most sensitive effect of perinatal TCDD exposure on the male reproductive system. Daily sperm production was decreased by ≤43 percent at puberty, day 49, but the decrease was less at sexual maturity, day 120. Seminiferous tubule diameter was decreased at all four developmental stages. Each effect of TCDD was dose related and in all cases a significant decrease was seen in

TABLE 5-5
Effects of In Utero and Lactational TCDD Exposure on Indices of Androgenic Status

Index	Lowest effective maternal dose (μg TCDD/kg)[a]	Maximum effect[b]
Anogenital distance	0.16 (days 1, 4)	21% decrease (day 1)
Time to testis descent	0.16	1.7 day delay
Plasma testosterone concentration	NS	69% decrease (day 32)
Plasma 5α-dihydrotestosterone concentration	NS	59% decrease (day 49)
Plasma LH concentration	1.0 (day 32)	95% decrease (day 32)
Absolute seminal vesicle weight	0.16 (days 32, 63)	56% decrease (day 49)
Relative seminal vesicle weight[c]	0.16 (day 63)	50% decrease (day 49)
Absolute ventral prostate weight	0.064 (day 32)	60% decrease (day 32)
Relative ventral prostate weight[c]	0.16 (days 32, 63)	53% decrease (day 32)

[a]The lowest dose of TCDD (given on day 15 of gestation) that caused a significant ($p < 0.05$) effect in the male offspring and the day or days at which this dose caused such an effect are shown.
[b]The magnitude of the greatest change seen in response to maternal dosing with 1.0 μg TCDD/kg and the day at which this effect was seen are shown.
[c]Weight of organ divided by body weight of rat.
NS = not statistically significant.

Source: Mably et al., 1991.

response to the lowest maternal TCDD dose tested, 0.064 μg/kg, during at least one stage of sexual development. In general, the magnitude of the decreases recovered with time, though not completely, suggesting that perinatal TCDD exposure delays sexual maturation. These results are summarized in Table 5-6 (Mably et al., 1991, 1992c). Gray et al. (1993) reported similar findings with respect to daily sperm production and epididymal sperm count but did not observe a statistically significant reduction in either paired testes weight or caudal epididymal weight.

Severe preweaning and/or postweaning undernutrition can affect the reproductive system of adult male rodents, including decreased spermatogenesis (Ghafoorunissa, 1980; Jean-Faucher et al., 1982a,b; Glass et al., 1986). At the two highest maternal TCDD doses in the Mably et al. (1992a) study, the feed consumption and body weight of male offspring were decreased such that the decreases were not more than 21 percent of control values. However, reduction in sex organ weights, epididymal sperm reserves, and spermatogenesis occurred at the two lowest maternal TCDD doses, neither of which reduced feed intake or body weight of the male offspring. Thus, undernutrition cannot account for these reproductive system effects, including the decreases in spermatogenesis observed at the lower maternal TCDD doses (Mably et al., 1992a,c).

Because FSH and testosterone are essential for quantitatively normal spermatogenesis (Steinberger and Steinberger, 1989), an alternative explanation for the decreases in daily sperm production is a decrease in FSH and/or testosterone levels. In rats, the duration of spermatogenesis is 58 days (Blazak et al., 1985; Amann, 1986; Working and Hurtt, 1987), so the decreases in plasma FSH concentrations in 32-day-old male offspring could contribute to the reductions of spermatogenesis when the rats were 49 and 63 days of age. However, the modest depressant effect of perinatal TCDD exposure on plasma FSH concentrations was transitory; no effect was found on plasma FSH levels when the offspring were 49, 63, and 120 days old. It was concluded that reduced spermatogenesis in 120-day-old male rats, perinatally exposed to TCDD, is not due to decreases in plasma FSH levels when the animals were 49 to 120 days of age (Mably et al., 1992c). Likewise, even if the original findings of

TABLE 5-6
Effects of *In Utero* and Lactational TCDD Exposure on Indices of Spermatogenic Function and Reproductive Capability

Index	Lowest effective maternal dose (μg TCDD/kg)[a]	Maximum effect[b]
Testis weight	0.40 (days 32)	17% decrease (day 32)
Epididymis weight	0.064 (days 49, 120)	35% decrease (day 32)
Cauda epididymis weight	0.064 (days 63, 120)	53% decrease (day 63)
Sperm per cauda epididymis	0.064 (days 63, 120)	75% decrease (day 63)
Daily sperm production rate	0.064 (days 63, 120)	43% decrease (day 49)
Seminiferous tubule diameter	0.064 (days 32, 49, 120)	15% decrease (day 32)
Plasma FSH concentration	0.40 (day 32)	15% decrease (day 32)
Leptotene spermatocyte: Sertoli cell ratio	NS	No dose-related effects
Sperm motility; percentage abnormal sperm	NS	No dose-related effects
Fertility	NS	22% decrease (day 70)
Gestation index; litter size; live birth index; pup survival	NS	No dose-related effects

[a]The lowest dose of TCDD (given on day 15 of gestation) that caused a significant ($p < 0.05$) effect in the male offspring and the day or days at which this dose caused such an effect are shown.
[b]The magnitude of the greatest change seen in response to maternal dosing with 1.0 μg TCDD/kg and the day at which this effect was seen are shown.
NS = not statistically significant.

Source: Mably et al., 1991.

Mably et al. (1992) are confirmed, the potential reduction in intratesticular testosterone levels following TCDD exposure would not be sufficient to reduce spermatogenesis (Zirkin et al., 1989; Mably et al., 1992c; Gray et al., 1993).

In normal rats, daily sperm production does not reach a maximum until 100 to 125 days of age (Robb et al., 1978), but in rats perinatally exposed to TCDD it takes longer for sperm production to reach the adult level. Furthermore, the length of the delay is directly related to maternal TCDD dose (Mably et al., 1992c), and if the dose is high enough, the reduction in spermatogenesis may be permanent. This is suggested by a maternal TCDD dose of 1.0 μg/kg decreasing daily sperm production in male rat offspring that are 300 days of age (Moore et al., 1992). Since the mechanism by which perinatal TCDD exposure decreases spermatogenesis in adulthood is unknown, it is unclear whether the irreversible effect at the largest maternal dose of 1 μg/kg, which results in depressed feed consumption and decreased body weight, is caused by the same mechanism as that at smaller maternal doses, which do not result in undernutrition and from which the male offspring may eventually recover.

A key observation for postulating mechanisms by which perinatal TCDD exposure reduces spermatogenesis in adulthood is the finding that the ratio of leptotene spermatocytes per Sertoli cell in the testes of 49-, 63-, and 120-day-old rats is not affected by in utero and lactational TCDD exposure even though daily sperm production is reduced (Mably et al., 1992c). Since Sertoli cells provide spermatogenic cells with functional and structural support (Bardin et al., 1988) and the upper limit of daily sperm production in adult rats is directly dependent on the number of Sertoli cells per testis (Russell and Peterson, 1984), three possible mechanisms for the decrease in daily sperm production may be involved. TCDD could increase the degeneration of cells intermediate in development between leptotene spermatocytes and terminal stage spermatids (the cell type used to calculate daily sperm production); decrease postleptotene spermatocyte cell division (meiosis); and/or decrease the number of Sertoli cells per testis (Orth et al., 1988). Elucidating the mechanism by which

perinatal TCDD exposure decreases spermatogenesis is important because it is one of the most sensitive responses of the male reproductive system to TCDD.

5.2.3.1.4. ***Epididymis.*** The epididymis has two functions: In proximal regions, spermatozoa mature gaining the capacity for motility and fertility, whereas in distal regions mature sperm are stored before ejaculation (Robaire and Hermo, 1989). Mably et al. (1991, 1992c) found that motility and morphology of sperm taken from the cauda epididymis on postnatal days 63 and 120 were unaffected by perinatal TCDD exposure. Thus, no effect of TCDD on epididymal function was detected. The dose-dependent reduction in epididymis and cauda epididymis weights in postpubertal rats, 63 and 120 days old, can be accounted for, in part, by decreased sperm production. However, in immature males, 32 and 49 days of age, where sperm are not present in the epididymis, the decrease in weights of epididymal tissue, as observed by Mably et al. (1992c), cannot be explained by effects on sperm production. Since epididymal growth is androgen dependent, a TCDD-induced androgenic deficiency and/or decrease in androgen responsiveness of the epididymis could account for decreased size of the organ (Setty and Jehan, 1977; Dhar and Setty, 1990).

5.2.3.1.5. ***Reproductive capability.*** To assess reproductive capability, male rats born to dams given TCDD (0.064, 0.16, 0.40, or 1.0 μg/kg) or vehicle on day 15 of gestation were mated with control virgin females when the males were ~70 and 120 days of age (Mably et al., 1991, 1992c). The fertility index of the males is defined as number of males impregnating females divided by number of males mated. The two highest maternal TCDD doses decreased the fertility index of the male offspring by 11 and 22 percent, respectively. However, these decreases were not statistically significant, and at lower doses, the fertility index was not reduced. Gestation index, defined as the percentage of control dams mated with TCDD-exposed males that delivered at least one live offspring, was also not affected by perinatal TCDD exposure. With respect to progeny of these matings, there was no effect on litter size, live birth index, or 21-day survival index. When perinatal TCDD-exposed males

were mated again at 120 days of age, there was no effect on any of these same parameters. Thus, despite pronounced reductions in cauda epididymal sperm reserves, when the TCDD-treated males were mated, perinatal TCDD exposure had little or no effect on fertility of male rats or on survival and growth of their offspring. These results are summarized in Table 5-6 (Mably et al., 1991, 1992c).

Since rats produce and ejaculate 10 times more sperm than is necessary for normal fertility and litter size (Aafjes et al., 1980; Amann, 1982), the absence of a reduction in fertility of male rats exposed perinatally to TCDD is not inconsistent with the substantial reductions in testicular spermatogenesis and epididymal sperm reserves. In contrast, reproductive efficiency in human males is very low, the number of sperm per ejaculate being close to that required for fertility (Working, 1988). Thus, measures of fertility using rats are not appropriate for low-dose extrapolation in humans (Meistrich, 1992). A percentage reduction in daily sperm production in humans, similar in magnitude to that observed in rats (Mably et al., 1991, 1992c) may reduce fertility in men.

5.2.3.1.6. ***Sexual differentiation of the CNS.*** Sexual differentiation of the CNS is dependent on the presence of androgens during early development. In rats, the critical period of sexual differentiation extends from late fetal life through the first week of postnatal life (MacLusky and Naftolin, 1981). In the absence of adequate circulating levels of testicular androgen during this time, adult rats display high levels of feminine sexual behavior (e.g., lordosis), low levels of masculine sexual behavior, and a cyclic (i.e., feminine) pattern of LH secretion (Gorski, 1974; Barraclough, 1980). In contrast, perinatal androgen exposure of rats will result in the masculinization of sexually dimorphic neural parameters, including reproductive behaviors, regulation of LH secretion, and several morphological indices (Raisman and Field, 1973; Gorski et al., 1978). The mechanism by which androgens cause sexual differentiation of the CNS is not completely understood. In the rat, it appears that 17ß-estradiol, formed by the aromatization of testosterone within the

CNS, is one of the principal active steroids responsible for mediating sexual differentiation (McEwen, 1978); however, androgens also are involved.

5.2.3.1.6.1. ***Demasculinization of sexual behavior.*** Mably et al. (1991, 1992b) assessed sexually dimorphic functions in male rats born to dams given graded doses of TCDD or vehicle on day 15 of gestation. Masculine sexual behavior was assessed in male offspring at 60, 75, and 115 days of age by placing a male rat in a cage with a receptive control female and observing the first ejaculatory series and subsequent postejaculatory interval (see Table 5-7). The number of mounts and intromissions (mounts with vaginal penetration) before ejaculation was increased by a maternal TCDD dose of 1.0 μg/kg. The same males exhibited twelvefold and elevenfold increases in mount and intromission latencies, respectively, and a twofold increase in ejaculation latency. All latency effects were dose related and significant at a maternal TCDD dose as low as 0.064 μg/kg (intromission latency) and 0.16 μg/kg (mount and ejaculation latencies). Copulatory rates (number of mounts + intromissions/time from first mount to ejaculation) were decreased to less than 43 percent of the control rate. This effect on copulatory rates was dose related, and a statistically significant effect was observed at maternal TCDD doses as low as 0.16 μg/kg. Postejaculatory intervals were increased 35 percent above the control interval, and a statistically significant effect was observed at maternal doses of TCDD as low as 0.40 μg/kg. Collectively, these results demonstrate that perinatal TCDD exposure demasculinizes sexual behavior.

Because perinatal exposure to a maternal TCDD dose of 1.0 μg/kg has no effect on the open field locomotor activity of adult male rats (Schantz et al., 1991), the increased mount, intromission, and ejaculation latencies appear to be specific for these masculine sexual behaviors, not secondary to a depressant effect of TCDD on motor activity. The reported postpubertal plasma testosterone and DHT concentrations in litter mates of the rats evaluated for masculine sexual behavior were as low as 56 and 62 percent, respectively, of controls (Mably et al., 1991, 1992a). However, plasma testosterone concentrations that were

TABLE 5-7
Effects of In Utero and Lactational TCDD Exposure on Indices of Sexual Behavior and Regulation of LH Secretion in Adulthood

INDEX	LOWEST EFFECTIVE MATERNAL DOSE (μg TCDD/kg)[a]	MAXIMUM EFFECT[b]
Masculine Sexual Behavior[c]		
Mount latency	0.16	1,200% increase
Intromission latency	0.064	1,100% increase
Ejaculatory latency	0.16	97% increase
Number of mounts	0.064	130% increase
Number of intromissions	1.0	38% increase
Copulatory rate (mounts plus intromissions/minute)	0.16	43% decrease
Postejaculatory interval	0.40	35% increase
Feminine Sexual Behavior[d]		
Lordosis quotient[e]	0.16	300% increase
Lordosis intensity score	0.40	50% increase
Regulation of LH Secretion		
LH surge	0.40	460% increase[f]

[a]The lowest dose of TCDD (given on day 15 of gestation) that caused a significant ($p < 0.05$) effect in the male offspring is shown.
[b]The magnitude of the greatest change seen in response to maternal dosing with 1.0 μg TCDD/kg is shown (average of three trials for masculine behavior and two for feminine).
[c]Measured when the rats were −60, 75, and 115 days of age.
[d]Feminine sexual behavior was measured following castration, estrogen priming, and progesterone administration. The rats were 170 to 184 days old.
[e]Number of times lordosis was displayed in reponse to a mount, divided by the number of times each rat was mounted, times 100.
[f]Since control males do not secrete LH in response to progesterone, this percentage was calculated by comparing peak plasma LH concentrations in TCDD-exposed rats with plasma LH concentrations in control males at the same time after progesterone was administered.

Source: Mably et al., 1991.

only 33 percent of controls are still sufficient to masculinize sexual behavior of adult male rats (Demassa et al., 1977). Therefore, the modest reductions in adult plasma androgen concentrations following perinatal TCDD exposure were not of sufficient magnitude to demasculinize sexual behavior.

Reductions in perinatal androgenic stimulation can inhibit penile development and subsequent sensitivity to sexual stimulation in adulthood (Nadler, 1969; Södersten and Hansen, 1978). Therefore, the demasculinization of sexual behavior could, to some extent, be secondary to decreased androgen-dependent penile development. However, perinatal TCDD exposure had no effect on gross appearance of the rat penis. In addition, TCDD-exposed males exhibited deficits in such masculine sexual behaviors as mount latency and postejaculatory interval, which do not depend on stimulation of the penis for expression (Sachs and Barfeld, 1976). Thus, although some effects of TCDD, such as decreased copulatory rate and prolonged latency until ejaculation, could be due to reduced sensitivity of the penis to sexual stimulation, the twelvefold increase in mount latency and increase in postejaculatory interval cannot be explained by this mechanism.

5.2.3.1.6.2. ***Feminization of sexual behavior.*** Mably et al. (1991, 1992b) determined if the potential of adult male rats to display feminine sexual behavior was altered by perinatal TCDD exposure. Male offspring of dams treated on day 15 of gestation with various doses of TCDD up to 1 μg/kg or vehicle were castrated at ~120 days of age, and beginning at ~160 days of age were injected weekly for 3 weeks with 17ß-estradiol benzoate, followed 42 hours later by progesterone. Four to six hours after the progesterone injection at weeks 2 and 3, the male was placed in a cage with a sexually excited control stud male. The frequency of lordosis in response to being mounted by the stud male was increased from 18 percent (control) to 54 percent by the highest maternal TCDD dose, 1.0 μg/kg (Table 5-7). Lordosis intensity, scored after Hardy and DeBold (1972) as 1 for light lordosis, 2 for moderate lordosis, and 3 for a full spinal dorsoflexion, was increased in male rats by perinatal TCDD exposure. Both effects on lordosis behavior in males were dose related and

significant at maternal TCDD doses as low as 0.16 μg/kg (increased lordotic frequency) and 0.40 μg/kg (increased lordotic intensity). Together, they indicate a feminization of sexual behavior in these animals. Although severe undernutrition from 5 to 45 days after birth potentiates the display of lordosis behavior in adult male rats (Forsberg et al., 1985), the increased frequency of lordotic behavior was seen at a maternal TCDD dose of 0.16 μg/kg, which had no effect on feed intake or body weight. It was concluded that perinatal TCDD exposure feminizes sexual behavior in adult male rats independent of undernutrition.

5.2.3.1.6.3. ***Feminization of LH secretion regulation.*** The effect of perinatal TCDD exposure on regulation of LH secretion by ovarian steroids was determined in male offspring at ~270 days of age. There is normally a distinct sexual dimorphism to this response. In rats castrated as adults, estrogen-primed females greatly increase their plasma LH concentrations when injected with progesterone, whereas similarly treated males fail to respond (Taleisnik et al., 1969). Progesterone had little effect on plasma LH concentrations in estrogen-primed control males, but significant increases were seen in males exposed to maternal TCDD doses as low as 0.40 μg/kg. Thus, perinatal TCDD exposure increases pituitary and/or hypothalamic responsiveness of male rats to ovarian steroids in adulthood, indicating that regulation of LH secretion is permanently feminized. Table 5-7 summarizes sexual behavior and LH secretion results (Mably et al., 1991, 1992b).

5.2.3.1.6.4. ***Comparison to other Ah receptor-mediated responses.*** The induction of hepatic cytochrome P-4501A1 and its associated EROD activity are extremely sensitive Ah receptor-mediated responses to TCDD exposure. Yet in 120-day-old male rats that had been exposed to TCDD perinatally, alterations in sexual behavior, LH secretion, and spermatogenesis were observed when induction of hepatic EROD activity could no longer be detected (Mably et al., 1991, 1992a,b,c). These results suggest that TCDD affects sexual behavior, gonadotrophic function, and spermatogenesis when virtually no TCDD remains in the body and that the demasculinization and feminization of sexual behavior, feminization of

LH secretion, and reduced spermatogenesis caused by in utero and lactational exposure to TCDD may be irreversible (Mably et al., 1992b,c).

5.2.3.1.6.5. ***Possible mechanisms and significance.*** The most plausible explanation for the demasculinization of sexual behavior and feminization of sexual behavior and LH secretion is that perinatal exposure to TCDD impairs sexual differentiation of the CNS. Neither undernutrition, altered locomotor activity, reduced sensitivity of the penis to sexual stimulation, nor modest reductions in adult plasma androgen concentrations of the male offspring can account for this effect (Mably et al., 1992b). On the other hand, exposure of the developing brain to testosterone, conversion of testosterone into 17ß-estradiol within the brain, and events initiated by the binding of 17ß-estradiol to its receptor are all critical for sexual differentiation of the CNS and have the potential to be modulated by TCDD. If TCDD interferes with any of these processes during late gestation and/or early neonatal life, it could irreversibly demasculinize and feminize sexual behavior (Hart, 1972; McEwen et al., 1977; Whalen and Olsen, 1981) and feminize the regulation of LH secretion (Gogan et al., 1980, 1981) in male rats in adulthood.

In utero and/or lactational exposure to TCDD may cause similar effects in other animal species, including nonhuman primates (Pomerantz et al., 1986; Thornton and Goy, 1986; Goy et al., 1988), in which sexual differentiation is under androgenic control. In humans, there is evidence that social factors account for much of the variation in sexually dimorphic behavior; there is also evidence that prenatal androgenization influences both the sexual differentiation of such behavior and brain hypothalamic structure (Erhardt and Meyer-Bahlburg, 1981; Hines, 1982; LeVay, 1991).

5.2.3.2. *Female Reproductive System*

Very little investigation on the developing female reproductive system, comparable to that on the male system, has been carried out. Gray et al. (1993) have begun to characterize the effects of a single maternal exposure (1 ug/kg on gestation day 15) on postnatal

development. This exposure was associated with clefting of the phallus/clitoris (with and without hypospadias), incomplete or absent vaginal opening, and a smaller vaginal orifice. There was no effect on estrous cyclicity from puberty to 5 months of age, and small effects on anogenital distance appeared to be associated with body weight changes. Mably et al. (1992a) reported a similar association of anogenital distance and crown-rump length in female offspring.

5.2.3.3. *Neurobehavior*

Because differentiated tissues derived from ectoderm, namely, skin, conjunctiva, nails, and teeth, are sites of action of halogenated aromatic hydrocarbons in transplacentally exposed human infants, another highly differentiated tissue derived from ectoderm, the CNS, should be considered a potential site of TCDD action. In support of this possibility, sexual differentiation of the CNS of adult male rats is irreversibly altered in a dose-related fashion by perinatal exposure to TCDD (Mably et al., 1991, 1992b). As will be shown below, the CNS of mice transplacentally exposed to 3,3',4,4'-TCB, monkeys perinatally exposed to TCDD, and children transplacentally exposed to a mixture of PCBs, CDFs, and PCQs in the Yu-Cheng incident is also affected. Thus, functional CNS alterations, which may or may not be irreversible, are observed following perinatal exposure to halogenated aromatic hydrocarbons. Ah receptors have been identified in rat brain (Carlstedt-Duke, 1979) but may be associated with glial cells rather than neurons (Silbergeld, 1992). Following administration of ^{14}C-TCDD in the rat, the highest concentrations of TCDD-derived ^{14}C are found in the hypothalamus and pituitary. Much lower concentrations are found in the cerebral cortex and cerebellum (Pohjanvirta et al., 1990). In another study, the Ah receptor was not detected in whole rat or mouse brain but was detected in the cerebrum of the hamster and cerebrum and cerebellum of the guinea pig (Gasiewicz, 1983). Ah receptors appear to be absent in the human frontal cortex (Silbergeld, 1992).

5.2.3.3.1. ***Mice.*** CD-1 mice exposed transplacentally to 3,3′,4,4′-TCB at a maternal oral dose of 32 mg/kg administered on days 10 to 16 of gestation exhibited neurobehavioral, neuropathological, and neurochemical alterations in adulthood (Tilson et al., 1979; Chou et al., 1979; Agrawal et al., 1981). The neurobehavioral effects consisted of circling, head bobbing, hyperactivity, impaired forelimb grip strength, impaired ability to traverse a wire rod, impaired visual placement responding, and impaired learning of a one-way avoidance task (Tilson et al., 1979). The brain pathology in adult mice exhibiting this syndrome consisted, in part, of alterations in synapses of the nucleus accumbens (Chou et al., 1979). This suggested that in utero exposure to 3,3′,4,4′-TCB may interfere with synaptogenesis of dopaminergic systems. In support of this possibility, Agrawal et al. (1981) found that adult mice transplacentally exposed to 3,3′,4,4′-TCB had decreased dopamine levels and decreased dopamine receptor binding in the corpus striatum, both of which were associated with elevated levels of motor activity. It was concluded that transplacental exposure to 3,3′,4,4′-TCB in mice may permanently alter development of striatal synapses in the brain.

Eriksson (1988) examined the neurobehavioral effects of 3,3′,4,4′-TCB in NMRI mice exposed to a single oral dose of 0.41 or 41 mg/kg on postnatal day 10. Following sacrifice of the mice on day 17, muscarinic receptor concentrations in the brain were significantly decreased at both dose levels. This effect was shown to occur in the hippocampus but not in the cortex. More recently (Eriksson et al., 1991), NMRI mice were exposed to the same two doses of 3,3′,4,4′-TCB similarly administered on postnatal day 10. At 4 months of age, the effects of the PCB on locomotor activity were assessed. At both dose levels, abnormal activity patterns were exhibited in that the treated mice were significantly less active than controls at the onset of testing, but were more active than controls at the end of the test period. This pattern of effects can be interpreted as a failure to habituate to the test apparatus. In contrast to the previous results with CD-1 mice, circling or head bobbing activities were not observed in these animals. Upon sacrifice after the activity testing was complete, a small but statistically significant increase (as opposed to the decrease found after sacrifice on postnatal day 17) in the muscarinic receptor

concentration of the hippocampus was found in animals from the high-dose group. These results suggest that the neurochemical effects of 3,3′,4,4′-TCB are complex. Cholinergeric as well as dopaminergic systems in the brain are involved.

Of all the developmental and reproductive endpoints reported in this chapter for laboratory animals, the only ones that have not yet been demonstrated to occur following perinatal exposure to TCDD are the above neurotoxic effects in mice. These have only been studied following perinatal exposure to 3,3′4,4′-TCB. In addition, there is as yet no evidence to show (1) that among inbred mouse strains having low- and high-affinity Ah receptors, susceptibility to 3,3′,4,4′-TCB-induced neurotoxicity segregates with the Ah locus or (2) that the rank order binding affinity of congeners for the Ah receptor correlates with their rank order potency for causing these neurotoxic effects in mice. The rapid metabolism of 3,3′,4,4′-TCB compared with the relatively slow metabolism of TCDD in mice causes some uncertainty about the potential involvement of the Ah receptor in 3,3′,4,4′-TCB-induced neurotoxicity. Contributing to this uncertainty is the hypothesis that 3,3′,4,4′-TCB might produce central nervous system effects by being converted to a hydroxylated metabolite that is neurotoxic. Although there is no evidence for or against this hypothesis, there is also no evidence for or against the Ah receptor mechanism hypothesis of 3,3′,4,4′-TCB neurotoxicity. Further research is needed to test these hypotheses. In so doing, it should become apparent whether 3,3′,4,4′-TCB-induced neurotoxicity effects are relevant to TCDD-induced developmental toxicity.

5.2.3.3.2. ***Monkeys.*** Schantz and Bowman (1989) and Bowman et al. (1989a) have conducted a series of studies on the long-term behavioral effects of perinatal TCDD exposure in monkeys. Because these were the first studies to evaluate the behavioral teratology of TCDD, monkeys exposed to TCDD via the mother during gestation and lactation were screened on a broad selection of behavioral tests at various stages of development (Bowman et al., 1989a). At the doses studied (5 or 25 ppt in the maternal diet), TCDD did not affect reflex development, visual exploration, locomotor activity, or fine motor control in any

consistent manner (Bowman et al., 1989b). However, the perinatal TCDD exposure did produce a specific, replicable deficit in cognitive function (Schantz and Bowman, 1989). TCDD-exposed offspring were impaired on object learning, but were unimpaired on spatial learning. TCDD exposure also produced changes in the social interactions of mother-infant dyads (Schantz et al., 1986). TCDD-exposed infants spent more time in close physical contact with their mothers. The pattern of effects was similar to that seen in lead-exposed infants and suggested that mothers were providing increased care to the TCDD-exposed infants (Schantz et al., 1986).

5.2.3.3.3. ***Humans.*** The intellectual and behavioral development of Yu-Cheng children transplacentally exposed to PCBs, CDFs, and PCQs was studied through 1985 by Rogan et al. (1988). In Yu-Cheng children matched to unexposed children of similar age, area of residence, and socioeconomic status, there was a clinical impression of developmental or psychomotor delay in 12 (10 percent) Yu-Cheng children compared with 3 (3 percent) control children and of a speech problem in 8 (7 percent) Yu-Cheng children versus 3 (3 percent) control children. Also, except for verbal IQ on the Wechsler Intelligence Scale for Children, Yu-Cheng children scored lower than control children on three developmental and cognitive tests (Rogan et al., 1988). Neurobehavioral data on Yu-Cheng children obtained after 1985 shows that the intellectual development of these children continues to lag somewhat behind that of matched control children. In addition, Yu-Cheng children are rated by their parents and teachers as having a higher activity level; more health, habit, and behavioral problems; and a temperamental clustering closer to that of a "difficult child." It is concluded that in humans, transplacental exposure to halogenated aromatic hydrocarbons can affect CNS function postnatally. However, which congeners, TCDD-like versus non-TCDD-like, are responsible for the neurotoxicity is unknown.

Further research on the mechanism of these postnatal neurobehavioral effects, dose-response relationships, and reversibility of the alterations is needed before the role of TCDD-like congeners versus non-TCDD-like congeners in causing this toxicity can be understood.

Mechanisms that respond uniquely to TCDD-like congeners may not necessarily be involved, as three lightly chlorinated, ortho-substituted PCB congeners, 2,4,4′-TCB, 2,2′,4,4′-TCB, and 2,2′,5,5′-TCB, have been detected in monkey brain following dietary exposure to Aroclor 1016 and appear to be responsible for decreasing dopamine concentrations in the caudate, putamen, substantia nigra, and hypothalamus of these animals (Seegal et al., 1990). These nonplanar PCB congeners are believed to cause these effects by acting through a mechanism that does not involve the Ah receptor. On the other hand, the results presented for mice and monkeys suggest that TCDD-like congeners could be involved in producing the observed postnatal neurobehavioral effects in humans.

5.2.4. Cross-Species Comparison of Effect Levels

TCDD exposure levels that cause a variety of developmental effects in different species are summarized for fish in Table 5-8, birds in Table 5-9, and mammals in Table 5-10. Fertilized lake trout eggs and Japanese medaka eggs were exposed to different waterborne concentrations of ^{3}H-TCDD. Estimates of the amount of TCDD in these eggs were then made from measurement of the TCDD-derived radioactivity within them. Fertilized rainbow trout, chicken, ring-necked pheasant, and eastern bluebird eggs were injected directly with the indicated doses of TCDD. Thus, the doses of TCDD given in Tables 5-8 and 5-9 for all fish and bird species represent TCDD egg burdens where a significant portion of the dose may be present within the yolk of the egg rather than the developing embryo.

Mammalian embryo/fetuses, on the other hand, were exposed via administration of TCDD to the pregnant female. Therefore, the doses given in Table 5-10 are maternal TCDD doses, where a significant portion of the dose may be retained by the mother and never actually reach the embryo/fetus. In some studies, pregnant rats and rhesus monkeys were exposed to TCDD on a chronic or subchronic basis, respectively. The doses given in Table 5-10 for these particular studies represent the calculated maternal body burdens at the time of conception. In rats, the duration of chronic exposure was much longer than the whole body

TABLE 5-8
Cross-Species Comparison of NOAELs and LOAELs for TCDD Developmental Toxicity in Fish

Species	Effect	Exposure	Egg dose	Effect level	Reference
Lake trout	Sac fry mortality	Static waterborne	34 ng/kg	NOAEL	Walker et al., 1991
Japanese medaka	Lesions[a]	Static waterborne	<100 ng/kg	NOAEL	Wisk and Cooper, 1990a
Rainbow trout	Sac fry mortality	Single injection	194 ng/kg	NOAEL	Walker et al., 1992
Lake trout	Sac fry mortality Sac fry mortality	Static waterborne Static waterborne	40 ng/kg 55 ng/kg	LOAEL LOAEL	Spitsbergen et al., 1991 Walker et al., 1991
Rainbow trout	Sac fry mortality	Single injection	291 ng/kg	LOAEL	Walker et al., 1992
Japanese medaka	Lesions[a]	Static waterborne	300 ng/kg	LOAEL	Wisk and Cooper, 1990a

[a]Consist of a spectrum of effects including hemorrhage in various areas, pericardial edema, collapse of the yolk sphere, cessation of blood flow throughout the animal, and embryo mortality.

TABLE 5-9
Cross-Species Comparison of NOAELs and LOAELs for TCDD Developmental Toxicity in Birds

Species	Effect	Exposure	Egg dose	Effect level	Reference
Ring-necked pheasant	Embryo mortality	Single injection	100 ng/kg	NOAEL	Nosek et al., 1993
Eastern bluebird	Embryo mortality	Single injection	1,000 ng/kg	NOAEL	Thiel et al., 1988 Martin et al., 1989
Chicken	Cardiac malformations	Single injection	9 ng/kg[a]	LOAEL	Cheung et al., 1981a,b
Chicken	Embryo mortality	Single injection	240 ng/kg	LD_{50}	Allred and Strange, 1977
Ring-necked pheasant	Embryo mortality	Single injection	1,000 ng/kg	LOAEL	Nosek et al., 1993
Ring-necked pheasant	Embryo mortality	Single injection	1,354 ng/kg[b]	LD_{50}	Nosek et al., 1993
Ring-necked pheasant	Embryo mortality	Single injection	2,182 ng/kg[c]	LD_{50}	Nosek et al., 1993
Eastern bluebird	Embryo mortality	Single injection	10,000 ng/kg	LOAEL	Thiel et al., 1988

[a]Chi-square analysis of the data in Table 1 of Cheung et al. (1981b) demonstrated that the incidence of cardiac malformations in all embryos examined at dose levels of 1.6 pmol/egg or greater are significantly ($p < 0.05$) increased compared to the incidence in the control group designated "all examined." Assuming a 55 g egg weight, 1.6 pmol/egg corresponds to a TCDD egg burden of 9 ng/kg.
[b]Injected into the egg albumin.
[c]Injected into the egg yolk.

TABLE 5-10
Cross-Species Comparison of NOAELs and LOAELs for TCDD Developmental Toxicity in Mammals

Species	Effect	Exposure	Maternal dose	Effect level	Reference
Monkey	Prenatal mortality	Multiple dose	22 ng/kg, 9x, gd 20-40	NOAEL	McNulty, 1984
Rat	Prenatal mortality	1 ng/kg/day	27 ng/kg[a], chronic	NOAEL	Murray et al., 1979
Rat	Prenatal mortality	Multiple dose	30 ng/kg/day, gd 6-15	NOAEL	Sparschu et al., 1971
Mouse	Hydronephrosis	Multiple dose	100 ng/kg/day, gd 6-15	NOAEL	Smith et al., 1976
Mouse	Cleft palate	Multiple dose	300 ng/kg/day, gd 6-15	NOAEL	Neubert and Dillman, 1972
Monkey	Object learning	0.126 ng/kg/day	19 ng/kg[a], subchronic	LOAEL	Schantz and Bowman, 1989
Rat	Male reproductive	Single dose	64 ng/kg, gd 15	LOAEL	Mably et al., 1992a,b,c
Monkey	Prenatal mortality	0.642 ng/kg/day Multiple dose	97 ng/kg[a], subchronic 111 ng/kg, 9x, gd 20-40	LOAEL LOAEL	Schantz and Bowman, 1989 McNulty, 1984
Rabbit	Extra ribs	Multiple dose	100 ng/kg/day, gd 6-15	LOAEL	Giavini et al., 1982b
Rat	Fetal growth	Multiple dose	125 ng/kg/day, gd 6-15	LOAEL	Sparschu et al., 1971
Rabbit	Prenatal mortality	Multiple dose	250 ng/kg/day, gd 6-15	LOAEL	Giavini et al., 1982b
Rat	Prenatal mortality	10 ng/kg/day Multiple dose	270 ng/kg[a], chronic 500 ng/kg/day, gd 6-15	LOAEL LOAEL	Murray et al., 1979 Sparschu et al., 1971
Mouse	Hydronephrosis	Multiple dose	500 ng/kg/day, gd 6-15	LOAEL	Silkworth et al., 1989
Guinea pig	Prenatal mortality	Single dose	1,500 ng/kg, gd 14	LOAEL	Olson and McGarrigle, 1992
Hamster	Thymic hypoplasia	Single dose	1,500 ng/kg, gd 7 or 9	LOAEL	Olson and McGarrigle, 1992
Mouse	Cleft palate	Multiple dose	3,000 ng/kg/day, gd 6-15	LOAEL	Courtney and Moore, 1971
Hamster	Prenatal mortality	Single dose	18,000 ng/kg/day, gd 7 or 9	LOAEL	Olson et al., 1990
Mouse	Prenatal mortality	Single dose	24,000 ng/kg/day, gd 6	LOAEL	Couture et al., 1990b

[a]Maternal body burdens of TCDD at the time of conception were calculated by assuming a one-compartment open model and half-life for whole body TCDD elimination of 400 days in the monkey (McNulty et al., 1982) and 23.7 days in the rat (Rose et al., 1976). A bioavailabilty of 86.1 percent was used in the monkey and rat (Rose et al., 1976). The daily dietary exposure levels in rhesus monkeys were approxiately 5 and 25 ppt at the NOAEL and LOAEL doses, respectively. Rhesus monkeys were exposed to these levels of TCDD for 7 months prior to conception. At this time (0.525 half-lives) the cumulative amount of TCDD in rhesus monkeys was 30.5 percent of the calculated steady-state level. Rats were exposed to the indicated daily doses of TCDD for a period of 90 days (3.8 half-lives) prior to conception. At this time the cumulative amount of TCDD in rats was 92.8 percent of the calculated steady-state level.

gd = gestational day.

elimination half-life for TCDD in rats. Therefore, the body burden of TCDD given for the rat is 92.8 percent of the calculated steady-state body burden. In rhesus monkeys, the half-life for whole body elimination of TCDD is longer than the duration of exposure prior to conception. Therefore, the steady-state body burdens that would be expected for rhesus monkeys exposed to the different levels of dietary TCDD intake are approximately three times greater than the maternal body burdens estimated at the time of conception (Table 5-10).

In both rats and rhesus monkeys, the maternal body burdens are calculated using a one-compartment open model, assuming 86.1 percent bioavailability for TCDD. The bioavailability used for TCDD was determined in rats (Rose et al., 1976). As no estimate for TCDD bioavailability has been reported in rhesus monkeys, the same 86.1 percent value was used. The whole body elimination half-life used for TCDD in the rat is 23.7 days (Rose et al., 1976). McNulty et al. (1982) estimated a half-life of approximately 1 year for TCDD elimination from adipose tissue in the rhesus monkey, and for calculation of the body burdens estimated in Table 5-10, this half-life was rounded to 400 days for whole body elimination. The maternal body burden given for chronic exposure in the rat was calculated from the data of Murray et al. (1979). The maternal body burden given for subchronic exposure in the rhesus monkey was calculated from data obtained from Dr. R. E. Bowman (personal communication), which included the daily dietary TCDD exposure level for each pregnant female used in the studies reported by Bowman et al. (1989a,b) and Schantz and Bowman (1989). Dr. Bowman's results indicate that the range of TCDD half-lives in these monkeys was 200 to 600 days, which is consistent with the results of McNulty et al. (1982). The body burdens estimated for rhesus monkeys used in these studies are averages based on the average daily TCDD consumption of all pregnant females used at a particular level of maternal TCDD exposure.

As summarized in Table 5-8, lake trout and rainbow trout sac fry and Japanese medaka embryos are similarly affected by a spectrum of lesions that includes hemorrhage, edema, collapse of the yolk sac, cessation of blood flow, and embryo mortality. Estimates

of the NOAEL and LOAEL are given in Table 5-8 for the appearance of these lesions in Japanese medaka embryos and for embryo mortality in the two trout species. Although fertilized lake trout eggs and Japanese medaka eggs were exposed to various TCDD concentrations dissolved in static water, and fertilized rainbow trout eggs were injected directly with TCDD, the egg doses given in Table 5-8 represent the concentration of TCDD within the eggs themselves. Therefore, the different NOAELs and LOAELs for developmental toxicity in different fish species probably represent species differences in susceptibility to TCDD-induced developmental toxicity rather than differences in method of TCDD exposure. Of the three fish species, lake trout sac fry are the most sensitive to TCDD-induced mortality. However, based on the LOAELs shown in Table 5-8, the difference in susceptibility between fish species may be less than tenfold.

Based on the LOAELs shown in Table 5-9, the sensitivity of different bird species to TCDD-induced embryo mortality varies by more than fortyfold. The chicken embryo is more susceptible to TCDD-induced mortality than are embryos of the ring-necked pheasant and eastern bluebird. In addition, chicken embryos are highly sensitive to the formation of TCDD-induced structural defects in the heart and aortic arch. The incidence of cardiac malformations in the chicken embryo is increased at an egg exposure level as low as 9 ng TCDD/kg egg. However, such cardiac malformations have not been found in any other bird species that has been examined.

Table 5-10 summarizes the levels of TCDD exposure that cause certain structural malformations, functional alterations, and prenatal mortality in the embryo/fetus of different mammalian species. Based on the LOAELs given in Table 5-10, functional alterations in learning behavior and the male reproductive system occur at lower TCDD doses than those required to produce structural malformations. Although TCDD-induced developmental toxicity has been extensively studied in mice and rats, the LOAELs in Table 5-10 indicate that the embryo/fetus of rodent species is generally not as sensitive to TCDD-induced prenatal mortality as is the embryo/fetus of the rhesus monkey. The sensitivity of the embryo/fetus to TCDD-induced prenatal mortality in different mammalian species varies

approximately 240-fold. This is in contrast to the 1,000- to 5,000-fold variation in the LD_{50} of TCDD when adult animals of these same species are exposed. The agreement between studies with respect to the LOAEL in Table 5-10 for prenatal mortality in rats and monkeys is particularly striking. The 500 ng/kg dose of TCDD on gestational days 6 to 15 that caused prenatal mortality in rats (Sparschu et al., 1971) agrees with the maternal TCDD body burden of 270 ng/kg calculated from the chronic exposure of rats by Murray et al. (1979) to within a factor of 2. Similarly, the TCDD dose of 111 ng/kg that was given to rhesus monkeys nine times during the first trimester of pregnancy (McNulty, 1984) agrees with the maternal body burden of 97 ng/kg that increased prenatal mortality in rhesus monkeys following subchronic dietary exposure (Schantz and Bowman, 1989).

5.3. REPRODUCTIVE TOXICITY

5.3.1. Female

5.3.1.1. *Reproductive Function/Fertility*

TCDD and its approximate isostereomers have been shown to affect female reproductive endpoints in a variety of animal studies. Among the effects reported are reduced fertility, reduced litter size, and effects on the female gonads and menstrual/estrous cycle. These studies are reviewed below. Other TCDD effects on pregnancy maintenance, embryo/fetotoxicity, and postnatal development are covered in Section 5.2 of this chapter.

The study by Murray et al. (1979) employed a multigenerational approach, examining the reproductive effects of exposure of male and female rats over three generations to relatively low levels of TCDD (0, 0.001, 0.01, and 0.1 μg/kg/day). There was variation in the fertility index in both the control and the exposed groups, and a lower than desirable number of impregnated animals in the exposed groups. Nevertheless, the results showed exposure-related effects on fertility, an increased time between first cohabitation and delivery, and a decrease in litter size. The effects on fertility and litter size were observed at 0.1 μg/kg/day in the F_0 generation and at 0.01 μg/kg/day in the F_1 and F_2 generations. Additionally, in a 13-week exposure to 1 to 2 μg/kg/day of TCDD in nonpregnant female

rats, Kociba et al. (1976) reported anovulation and signs of ovarian dysfunction, as well as suppression of the estrous cycle. However, at exposures of 0.001 to 0.01 μg/kg/day in a 2-year study, Kociba et al. (1978) reported no effects on the female reproductive system.

Allen and colleagues reported on the effects of TCDD on reproduction in the monkey (Allen et al., 1977, 1979; Barsotti et al., 1979; Schantz et al., 1979). In a series of studies, female rhesus monkeys were fed 50 or 500 ppt TCDD for ≤9 months. Females exposed to 500 ppt showed obvious clinical signs of TCDD toxicity and lost weight throughout the study. Five of the eight monkeys died within 1 year after exposure was initiated. Following 7 months of exposure to 500 ppt TCDD, seven of the eight females were bred to unexposed males. The remaining monkey showed such severe signs of TCDD toxicity that she was not bred due to her debilitated state. Of the seven females that were evaluated for their reproductive capabilities, only three were able to conceive and, of these, only one was able to carry her infant to term (Barsotti et al., 1979). When females exposed to 50 ppt TCDD in the diet were bred at 7 months, two of eight females did not conceive and four of six that did conceive could not carry their pregnancies to term. As one monkey delivered a stillborn infant, only one conception resulted in a live birth (Schantz et al., 1979). As described in an abstracted summary, these results at 50 and 500 ppt TCDD are compared with a group of monkeys given a dietary exposure to PBB (0.3 ppm, Firemaster FF-1) in which seven of seven exposed females were able to conceive, five gave birth to live, normal infants, and one gave birth to a stillborn infant (Allen et al., 1979). Although the effects at 500 ppt TCDD may be associated with significant maternal toxicity, this would not appear to be the case at the lower dose. After administration of 50 ppt TCDD, no overt effects on maternal health were observed, but the ability to conceive and maintain pregnancy was reduced (Allen et al., 1979).

In a similar series of experiments, female rhesus monkeys were fed diets that contained 0, 5, and 25 ppt TCDD (Bowman et al., 1989b; Schantz and Bowman, 1989). Reproductive function was not altered in the 5 ppt group, as seven of eight females mated to unexposed males after 7 months of dietary exposure to TCDD were able to conceive. Six of

these females gave birth to viable infants at term and one gave birth to a stillborn infant. This was not significantly different from the results of the control group, which was fed a normal diet that contained no TCDD. All seven of the monkeys in this control group were able to conceive and give birth to viable infants. The 25 ppt dietary exposure level, however, did affect reproductive function. Only one of the eight females in this group that was mated gave birth to a viable infant. As in the 50 ppt group from earlier studies, there were no serious health problems exhibited by any females exposed to 0, 5, or 25 ppt TCDD. Therefore, the results in the 25 and 50 ppt groups suggest that maternal exposure to TCDD before and during pregnancy can result in fetomortality without producing overt toxic effects in the mother.

McNulty (1984) examined the effect of a TCDD exposure during the first trimester of pregnancy (gestational age 25 to 40 days) in the rhesus monkey. At a total dose of 1 μg/kg given in nine divided doses, three of four pregnancies ended in abortion and two of these abortions occurred in animals that displayed no maternal toxicity. At a total dose of 0.2 μg/kg, one of four pregnancies ended in abortion. This did not appear to be different from the control population, but the low number of animals per group did not permit statistical analysis. McNulty (1984) also administered single 1 μg/kg doses of TCDD on gestational days 25, 30, 35, or 40. The number of animals per group was limited to three, but it appeared that the most sensitive periods were the earlier periods, days 25 and 30, and that both maternal toxicity and fetotoxicity were reduced when TCDD was given on later gestational days. For all days at which a single 1 μg TCDD/kg dose was given (gestational day 25, 30, 35, or 40), 10 of 12 pregnancies terminated in abortion. Thus, of 16 monkeys given 1 μg TCDD/kg in single or divided doses between days 25 and 40 of pregnancy, only three normal births occurred (McNulty, 1984, 1985).

Of increasing interest is the recent report that TCDD exposure is associated with the appearance of endometriosis (Rier et al., 1993). Endometriosis is characterized by endometrial cell growth outside the uterus and can be associated with infertility and pain. Rier et al. (1993) determined the incidence of endometriosis in a colony of rhesus monkeys

that had been chronically exposed to TCDD 10 years earlier. They reported a 43-percent and a 71-percent incidence at 5 ppt and 25 ppt, respectively, compared with a control incidence of 33 percent. Moreover, the severity of endometriosis was dose dependent. Other studies are now under way to further examine the relationship of endometriosis following exposure to dioxin or dioxin-like compounds.

In conclusion, the primary effects of TCDD on female reproduction appear to be decreased fertility, inability to maintain pregnancy for the full gestational period, and in the rat, decreased litter size. In some studies, signs of ovarian dysfunction such as anovulation and suppression of the estrous cycle have been reported (Kociba et al., 1976; Barsotti et al., 1979; Allen et al., 1979). Unfortunately, the amount of attention that has been given to the female reproductive system, especially in the nonpregnant state, has been limited. In addition, there is little information on how TCDD toxicity involving the mother and/or placenta might affect fetal development.

5.3.1.2. *Alterations in Hormone Levels*

The potential for TCDD to alter circulating female hormone levels has been examined, but only to a very limited extent. In monkeys fed a diet that contained 500 ppt TCDD for ≤ 9 months, the length of the menstrual cycle, as well as the intensity and duration of menstruation, were not appreciably affected by TCDD exposure (Barsotti et al., 1979). However, there was a decrease in serum estradiol and progesterone concentration in five of the eight exposed monkeys, and in two of these animals the reduced steroid concentrations were consistent with anovulatory menstrual cycles. In summary form, Allen et al. (1979) described the effects of dietary exposure of female monkeys to 50 ppt TCDD. After 6 months of exposure to this lower dietary level of TCDD, there was no effect on serum estradiol and progesterone concentrations in these monkeys. Thus, the presence of these hormonal alterations is dependent on the level of dietary TCDD exposure. Shiverick and Muther (1983) reported that there was no change in circulating levels of estradiol in the rat after exposure to 1 μg/kg/day on gestational days 4 to 15. Taking all of these results

together, the effect of TCDD exposure on circulating female hormone levels may depend both on species and level of exposure. It appears that any significant effect is only seen at relatively high exposure levels, but very little research has been done and the studies to date have not been specifically designed to carefully examine alterations in female hormones.

5.3.1.3. *Antiestrogenic Action*

5.3.1.3.1. ***In vivo.*** Estrogens are necessary for normal uterine development and for maintenance of the adult uterus. The cyclic production of estrogens partially regulates the cyclic production of FSH and LH that results in the estrous cycling of female mammals. In addition, estrogens are necessary for the maintenance of pregnancy. Any effect that causes a decrease in circulating or target cell estrogen levels can alter normal hormonal balance and action.

Early experimental results in rats and monkeys indicated that TCDD may have an antiestrogenic action. Following administration of 1 μg TCDD/kg/day to rats for 13 weeks, Kociba et al. (1976) reported morphologic changes in the ovaries and uterus that were interpreted as being due to a suppression or inhibition of the estrous cycle. Rhesus monkeys exposed to 500 ppt of TCDD in the diet for 6 months developed hormonal irregularities in their estrous cycles that were associated with reduced conception rates as well as a high incidence of early spontaneous abortions (Allen et al., 1977; Barsotti et al., 1979).

In rhesus monkeys, the severity of the TCDD-associated reproductive alterations was correlated with decreased plasma levels of estrogen and progesterone (Barsotti et al., 1979). Thus, one possible mechanism for these effects would be increased metabolism of estrogen and progesterone due to induction by TCDD of hepatic microsomal enzymes and/or a decrease in the rate at which these steroids are synthesized. On the other hand, serum concentrations of 17β-estradiol are not significantly affected when TCDD is administered to pregnant rats (Shiverick and Muther, 1983). Thus, an alternative mechanism for TCDD-associated reproductive dysfunction could involve effects of TCDD on gonadal tissue itself, such as a decrease in its responsiveness to estrogen. In support of this latter mechanism, the

administration of TCDD to CD-1 mice results in a decreased number of cytosolic and nuclear estrogen receptors in hepatocytes and uterine cells. Although TCDD treatment induces hepatic cytochrome P-450 levels in these animals, it has no effect on serum concentrations of 17β-estradiol (DeVito et al., 1992). This indicates that the antiestrogenic effect of TCDD in CD-1 mice is not caused by a decrease in circulating levels of estrogen.

Effects of estrogen on the uterus include a cyclic increase in uterine weight, increased activity of the enzyme peroxidase, and an increase in the tissue concentration of progesterone receptors. Antiestrogenic effects of TCDD administration to female rats include a decrease in uterine weight, decrease in uterine peroxidase activity, and a decrease in the concentration of progesterone receptors in the uterus (Safe et al., 1991). In addition, when TCDD and 17β-estradiol are coadministered to the same female rat, the antiestrogenic action of TCDD diminishes or prevents 17β-estradiol-induced increases in uterine weight, peroxidase activity, progesterone receptor concentration, and expression of EGF receptor mRNA (Astroff et al., 1990; Safe et al., 1991). Similarly, in mice TCDD administration decreases uterine weight and antagonizes the ability of 17β-estradiol to increase uterine weight (Gallo et al., 1986).

The ability of TCDD to antagonize the effects of exogenously administered estrogen in the rat is dependent on the age of the animal. In 21-day-old rats, TCDD does not affect 17β-estradiol-induced increases in uterine weight or progesterone receptor concentration. On the other hand, in 28-day-old intact rats and 70-day-old ovariectomized rats, both of these 17β-estradiol-mediated responses are attenuated by TCDD (Safe et al., 1991). Previously, it had been reported that TCDD administration does not alter the dose-dependent increase in uterine weight due to exogenously administered estrone in sexually immature rats (Shiverick and Muther, 1982). The later work by Safe et al. (1991) suggests that this apparent lack of an antiestrogenic effect of TCDD may have been due to the young age of the rats used.

5.3.1.3.2. ***In vitro.*** Both TCDD and progesterone can affect a decrease in the nuclear estrogen receptor concentration in rat uterine strips (Romkes and Safe, 1988). However, the effect of progesterone is inhibited by actinomycin D, cycloheximide, and puromycin,

whereas the effect of TCDD is inhibited only by actinomycin D. The reasons that the TCDD-induced decrease in nuclear estrogen receptors is blocked by a transcription inhibitor, but not by protein synthesis inhibitors, are not understood. However, these results indicate that TCDD and progesterone decrease the nuclear estrogen receptor concentration by different mechanisms. In addition, the antiestrogenic actions of TCDD can be demonstrated in cell culture, and two prominent mechanisms could potentially be involved. They are (1) increased metabolism of estrogen due to Ah receptor-mediated enzyme induction and (2) a downregulation of estrogen receptors within the target cell.

In MCF-7 cells, which are estrogen-responsive cells derived from a human breast adenocarcinoma, antiestrogenic effects caused by the addition of TCDD to the culture medium include a reduction of the 17β-estradiol-induced secretion of a 160 kDa protein, 52 kDa protein, and 34 kDa protein (Biegel and Safe, 1990). These last two proteins are believed to be procathepsin D and cathepsin D, respectively. In addition, treatment of MCF-7 cells with TCDD suppresses the 17β-estradiol-enhanced secretion of tPA and inhibits estrogen-dependent postconfluent cell proliferation (Gierthy et al., 1987; Gierthy and Lincoln, 1988). Thus, cultured MCF-7 cells have several estrogen-dependent responses that are inhibited by TCDD; this characteristic makes them a useful model system for studying the antiestrogenic actions of dioxin.

In cultured MCF-7 cells, TCDD treatment induces aryl hydrocarbon hydroxylase (AHH) activity, the hallmark response of Ah receptor binding, and increases hydroxylation of 17β-estradiol at the C-2, C-4, C-6α, and C-15α, positions (Spink et al., 1990). It turns out that the particular cytochrome P-450 that catalyzes the C-2, C-15α, and C-6α hydroxylations of 17β-estradiol is cytochrome P-4501A1, which is identical to AHH (Spink et al., 1992). TCDD treatment also results in reduced levels of occupied nuclear estrogen receptors (Harris et al., 1990). These results indicate, in MCF-7 cells, that the antiestrogenic effect of TCDD could result from (1) an increased metabolism of estrogens due to Ah receptor-mediated enzyme induction and/or (2) a decreased number of estrogen receptors in the nucleus. Safe and his colleagues have published TCDD-concentration-

response information for both the TCDD-induced decrease in occupied nuclear estrogen receptors (Harris et al., 1989) and the induction of AHH and EROD activities in MCF-7 cells (Harris et al., 1990). In addition, they have reported that TCDD causes a decreased number of cytosolic and nuclear estrogen receptors in Hepa 1c1c7 cells, which are a mouse hepatoma cell line (Zacharewski et al., 1991).

Independent analysis of the data suggests that the EC_{50} values for these effects are not dissimilar enough to distinguish between the proposed mechanisms. Instead, it appears as though TCDD induces the enzymes AHH and EROD over the same concentration range that it causes a decreased concentration of occupied nuclear estrogen receptors in MCF-7 cells. In Hepa 1c1c7 cells, the lowest concentration used was 10 pM. Although exposure to 10 pM TCDD resulted in a statistically significant downregulation of estrogen receptors, Israel and Whitlock (1983) reported that this concentration is the approximate EC_{50} for the induction of cytochrome P-4501A1 mRNA and enzyme activity in these cells. Therefore, in Hepa 1c1c7 cells as well as in MCF-7 cells, it would appear that the TCDD concentrations required to produce enzyme induction and reduction in occupied nuclear estrogen receptor levels are not dissimilar enough to distinguish between the two proposed mechanisms.

More recently, Safe and his colleagues have used an analog of TCDD, MCDF, which inhibits the 17β-estradiol-induced secretion of the 34, 52, and 160 kDa proteins and downregulates estrogen receptors in MCF-7 cells. These effects occurred at concentrations of MCDF at which there is no detectable induction of EROD activity (Zacharewski et al., 1992). In addition, it has been stated that the downregulation of estrogen receptors in Hepa 1c1c7 cells can be detected as early as 1 hour after exposure of the cell cultures to 10 nM TCDD (Zacharewski et al., 1991). This time is slightly less than the 2 hours that was required for Israel and Whitlock (1983) to detect an increase in cytochrome P-450IA1 mRNA levels after exposure of Hepa 1c1c7 cells to 10 pM TCDD. After exposure of Hepa 1c1c7 cells to a maximally inducing concentration of 1 nM TCDD, however, there are significant increases in the cellular concentration of cytochrome P-450IA1 mRNA after 1 hour, whereas the induction of AHH activity takes slightly longer (Israel and Whitlock, 1983).

Gierthy et al. (1987) reported that exposure of MCF-7 cells to 1 nM TCDD caused suppression of the 17β-estradiol-induced secretion of tPA. This effect of TCDD, however, occurred in the absence of any measurable decrease in the whole cell concentration of estrogen receptors, even though the cultures were pretreated with serum-free medium to reduce cell proliferation and maximize the cellular content of estrogen receptors. Gierthy's group pretreated their cultures with serum-free medium, which was done to reduce cell proliferation and maximize the cellular content of estrogen receptors. The disparity between this result of Gierthy et al. (1987), which suggests no effect of TCDD on the estrogen receptor content of MCF-7 cells, and the results of Safe and his colleagues to the contrary in this same cell line remains largely unexplained. Overall, it appears as though no obvious distinction between the two proposed mechanisms can be made at the present time. Therefore, it seems that the antiestrogenic effect of TCDD results from both an increased metabolism of estrogen and a decreased number of estrogen receptors. It is important to note that TCDD does not compete with radiolabeled estrogens or progesterone for binding to estrogen or progesterone receptors and that these steroids do not bind to the Ah receptor or compete with radiolabeled TCDD for binding (Romkes et al., 1987; Romkes and Safe, 1988).

5.3.1.3.3. ***Evidence for an Ah receptor mechanism.***

5.3.1.3.3.1. ***Ah receptor mutants.*** Although the precise cellular mechanism by which TCDD produces its antiestrogenic effect is subject to a discordance between two primary schools of thought, there is agreement that the response is mediated by the Ah receptor. Thus, the antiestrogenic effects of TCDD in cultured cells appear to involve an Ah receptor-mediated alteration in the transcription of genes. This is indicated by studies using wild-type Hepa 1c1c7 cells and mutant Hepa 1c1c7 cells in culture (Zacharewski et al., 1991). In wild-type cells, TCDD reduces the number of nuclear estrogen receptors, and this response can be inhibited by cycloheximide and actinomycin D. However, in class 1 mutants, which have relatively low Ah receptor levels, TCDD has only a small effect. Similarly, in class 2

mutants, which have a defect in the accumulation of transcriptionally active nuclear Ah receptors, there was no effect of TCDD on the number of nuclear estrogen receptors. Taken together, these results indicate that the downregulation of estrogen receptors in Hepa 1c1c7 cells involves an Ah receptor-mediated effect on gene transcription. As previously noted, TCDD induces cytochrome P-4501A1 mRNA transcription and enzyme activity in Hepa 1c1c7 cells (Israel and Whitlock, 1983). This effect is also Ah receptor mediated (Nebert and Gielen, 1972).

5.3.1.3.3.2. ***Structure-activity relationships in vivo.*** The relative potencies of halogenated aromatic hydrocarbon congeners as inhibitors of uterine peroxidase activity in the rat are similar to their relative Ah receptor-binding affinities (Astroff and Safe, 1990). Only limited relative potency information is available for the reduction of hepatic and uterine estrogen receptor concentrations per se by these substances in rats. TCDD and 1,2,3,7,8-PeCDD both exhibit high affinity for the Ah receptor. At an 80 μg/kg dose of either of these two substances, hepatic estrogen receptor concentrations are reduced 42 and 41 percent, whereas uterine estrogen receptor concentrations are reduced 53 and 49 percent by TCDD and 1,2,3,7,8-PeCDD, respectively. On the other hand, 1,3,7,8-TCDD and 1,2,4,7,8-PeCDD bind less avidly to the Ah receptor. At a 400 μg/kg dose of either of these two substances, hepatic estrogen receptor concentrations are reduced 36 and 40 percent, whereas uterine estrogen receptor concentrations are reduced 21 and 24 percent by 1,3,7,8-TCDD and 1,2,4,7,8-PeCDD, respectively (Romkes et al., 1987). As the potency of these congeners for reducing estrogen receptor concentrations correlates with their Ah receptor-binding affinities, these in vivo results provide evidence that the antiestrogenic effect of TCDD is mediated by the Ah receptor.

5.3.1.3.3.3. ***Genetic evidence.*** Consistent with the interpretation based on structure-activity relationships, there is a greater reduction in the number of hepatic estrogen receptors when Ah^bAh^b C57BL/6 mice are exposed to TCDD than when Ah^dAh^d DBA/2 mice are similarly

exposed (Lin et al., 1991). To date, however, the antiestrogenic effects have not been studied in the progeny of test crosses between Ah^bAh^b and Ah^dAh^d mouse strains that respectively produce Ah receptors with high- or low-binding affinity for TCDD. Therefore, the potential segregation of the antiestrogenic effects of TCDD with the Ah locus has not been verified by the results of genetic crosses.

5.3.1.3.3.4. ***Structure-activity relationships in vitro.*** The Ah receptor is detectable in MCF-7 cells, and AHH as well as EROD activities are both inducible in these cells (Harris et al., 1989). The relative abilities of TCDD and other CDD, CDF, and PCB congeners to suppress 17β-estradiol-induced secretion of tPA by MCF-7 cells are consistent with the structure-activity relationships for other Ah receptor-mediated responses (Gierthy et al., 1987). In addition, the rank order of potency for several Ah receptor agonists in reducing nuclear estrogen receptors in MCF-7 cells is TCDD > 2,3,4,7,8-PeCDD > 2,3,7,8-TCDF > 1,2,3,7,9-PeCDD > 1,3,6,8-TCDF (Harris et al., 1990). The rank order of potency for these substances is consistent with their relative activities as Ah receptor agonists. These results in vitro support a role for the Ah receptor in the antiestrogenic actions of TCDD.

5.3.2. Male

5.3.2.1. *Reproductive Function/Fertility*

TCDD and related compounds decrease testis and accessory sex organ weights, cause abnormal testicular morphology, decrease spermatogenesis, and reduce fertility when given to adult animals in doses sufficient to reduce feed intake and/or body weight. Certain of these effects have been reported in chickens, rhesus monkeys, rats, guinea pigs, and mice treated with overtly toxic doses of TCDD, TCDD-like congeners, or toxic fat that was discovered later to contain TCDD (Allen and Lalich, 1962; Allen and Carstens, 1967; Khera and Ruddick, 1973; Kociba et al., 1976; Van Miller et al., 1977; McConnell et al., 1978; Moore et al., 1985; Chahoud et al., 1989; Morrisey and Schwetz, 1989). In testis of these different species, TCDD effects on spermatogenesis are characterized by loss of germ cells, the

appearance of degenerating spermatocytes and mature spermatozoa within the lumens of seminiferous tubules, and a reduction in the number of tubules containing mature spermatozoa (Allen and Lalich, 1962; Allen and Carstens, 1967; McConnell et al., 1978; Chahoud et al., 1989). The lowest cumulative dose of TCDD to decrease spermatogenesis in the rat was 1 μg/kg/day administered 5 days a week for 13 weeks (Kociba et al., 1976). With this dosage regimen, which resulted in a TCDD body burden of approximately 20 μg/kg at the end of the dosing period (Rose et al., 1976), body weights and feed consumption of the rats also were significantly depressed. Thus, suppression of spermatogenesis is not a highly sensitive effect when TCDD is administered to postweanling animals.

5.3.2.2. *Alterations in Hormone Levels*

The effects of TCDD on the male reproductive system are believed to be due in part to an androgenic deficiency. This deficiency is characterized in adult rats by decreased plasma testosterone and DHT concentrations, unaltered plasma LH concentrations, and unchanged plasma clearance of androgens and LH (Moore et al., 1985, 1989; Mebus et al., 1987; Moore and Peterson, 1988; Bookstaff et al., 1990a). The ED_{50} of TCDD for producing this effect in adult male rats on day 7 after dosing is 15 μg/kg (Moore et al., 1985), and it can be detected within 1 day of treatment. As described in the following sections, the cause of the androgenic deficiency is decreased testicular responsiveness to LH and increased pituitary responsiveness to feedback inhibition by androgens and estrogens (Moore et al., 1989, 1991; Bookstaff et al., 1990a,b; Kleeman et al., 1990).

5.3.2.3. *Target Organ Responsiveness*

5.3.2.3.1. ***Inhibition of testicular steroidogenesis.*** Testicular steroidogenesis occurs within Leydig cells and is regulated primarily by plasma LH concentrations (Payne et al., 1985; Hall, 1988). Binding of LH to the LH receptor causes cAMP and possibly other second messengers to be formed (Cooke et al., 1989). In response, cholesterol is rapidly

transported to the initial enzyme in the testosterone biosynthetic pathway, a cholesterol side chain cleavage enzyme, which is a cytochrome P-450 (cytochrome P-450_{scc}) located on the inner side of the inner mitochondrial membrane that converts cholesterol to pregnenolone. The mobilization of free cholesterol rather than its conversion to pregnenolone and other metabolites is generally considered to be the rate-limiting step in testicular steroidogenesis. TCDD inhibits testosterone biosynthesis, predominantly if not exclusively by inhibiting the mobilization of free cholesterol that acts as a substrate for cytochrome P-450_{scc} (Moore et al., 1991). Thus, in the testes of TCDD-treated rats, cholesterol is provided to the cytochrome P-450_{scc} enzyme at too slow a rate to maintain androgenic homeostasis, even when the plasma LH concentration characteristic of "normal" androgen levels is present.

5.3.2.3.2. ***Altered regulation of pituitary LH secretion.*** In TCDD-treated male rats, the expected increase in plasma LH concentration that would facilitate testicular compensation for the decreased plasma androgens does not occur (Moore et al., 1989; Ruangwises et al., 1991). The failure of the plasma LH concentration to rise appropriately is not caused by an increase in the plasma clearance of LH or by a decrease in the maximal rate of pituitary LH synthesis or secretion (Bookstaff et al., 1990a,b). Rather, TCDD alters the feedback regulation of LH secretion in male rats by increasing the potency of testosterone and its metabolites (DHT and 17β-estradiol) as inhibitors of LH secretion. The ED_{50} of TCDD for enhancing the testosterone-mediated inhibition of LH secretion 7 days after treatment is the same as its ED_{50} for causing the androgenic deficiency (15 μg/kg). Also, both responses are detected within 1 day of TCDD dosing and are fully developed after 7 days when the ED_{50}s were determined.

Decreased plasma androgen concentrations normally result in compensatory increases in both the number of pituitary GnRH receptors and the responsiveness of the pituitary to GnRH. TCDD treatment prevents the increases in GnRH receptor number and responsiveness that would be expected in the light of the decreased plasma androgen concentrations (Bookstaff et al., 1990b). The pituitary is thus a target organ for TCDD

because its responsiveness to hormones secreted by the testis (testosterone) and hypothalamus (GnRH) is altered by TCDD.

If the plasma LH concentrations in TCDD-treated rats did increase appropriately in response to decreased plasma androgens, it is expected that plasma androgens would return to normal levels (Kleeman et al., 1990). This is because the testes of TCDD-treated rats are capable of synthesizing more testosterone than is needed to maintain androgen concentrations in the physiological range, although this would require significantly elevated levels of LH in TCDD-treated rats. The fact that there is a testicular reserve capacity to provide for sufficient amounts of androgen synthesis, even when compromised, underscores the importance of the effects of TCDD on pituitary LH secretion in producing the effects of TCDD on plasma androgen concentrations.

5.3.2.3.3. ***Differential responsiveness of androgen target organs.*** The dose-related reductions in plasma testosterone and DHT concentrations in intact adult rats are accompanied by similar dose-related reductions (ED_{50} 15 μg TCDD/kg) in seminal vesicle and ventral prostate weights measured 7 days after dosing (Moore et al., 1985). In contrast, TCDD has no effect on accessory sex organ weights (or plasma androgen concentrations) in castrated adult rats implanted with either testosterone- or DHT-containing capsules (Moore and Peterson, 1988; Bookstaff et al., 1990a,b). As trophic responsiveness of the seminal vesicles and ventral prostate to testosterone and DHT are unaffected by postpubertal TCDD treatment, it follows that TCDD can increase responsiveness of the pituitary to androgens without affecting responsiveness of the accessory sex organs to androgens.

5.3.2.3.4. ***Relative sensitivity.*** The male reproductive system in rats is ~100 times more susceptible to TCDD toxicity when exposure occurs perinatally (ED_{50} for the most sensitive effects, 0.16 μg/kg) rather than in adulthood (ED_{50} for the most sensitive effects, 15 μg/kg). To illustrate this sensitivity, a single maternal TCDD dose as low as 0.064 μg/kg given on day 15 of gestation significantly decreases epididymis and cauda epididymis weights, cauda

epididymal sperm numbers, and daily sperm production in male offspring at various stages of sexual development. Decreases in ventral prostate weights in 32-day-old male offspring and in older males, increases in the number of mounts preceding ejaculation, and increases in intromission latency also are produced by maternal TCDD doses as low as 0.064 μg/kg. The 0.064 μg TCDD/kg dose is not maternally toxic and produces no signs of overt toxicity in male or female offspring. Other effects of perinatal exposure on the male reproductive system were detected at a maternal TCDD dose of 0.16 μg/kg or higher (Mably et al., 1991, 1992a,b,c). On the other hand, when exposure occurs in adulthood, relatively large doses in the overtly toxic range are required to cause decreases in spermatogenesis and in ventral prostate and caput epididymis weight (Kociba et al., 1976; Moore et al., 1985). Kociba et al. (1976) reported that accessory sex organ weights and spermatogenesis are decreased in rats following exposure to 1 μg TCDD/kg/day, 5 days per week for 13 weeks. Using the parameters for TCDD half-life and bioavailability in the rat determined by Rose et al. (1976), this dosage regimen results in a TCDD body burden of approximately 20 μg/kg at the end of the dosing period.

In adult rats, the most sensitive toxic responses to TCDD have been observed following long-term, low-level exposure. In a 3-generation reproduction study, Murray et al. (1979) reported that dietary administration of TCDD at doses as low as 0.01 μg/kg/day significantly affected reproductive capacity in female rats, with no effects seen at 0.001 μg/kg/day (NOAEL). The same NOAEL was found in a 2-year chronic toxicity and oncogenicity study in which an increased incidence of certain types of neoplasms was altered among rats given TCDD doses of 0.01 or 0.1 μg/kg/day (Kociba et al., 1978). Based on the pharmacokinetics of TCDD in the rat (Rose et al., 1976), the steady-state body burden of TCDD in these rats that were chronically dosed (>90 days) with either 0.01 or 0.001 μg TCDD/kg/day is approximately 0.29 μg/kg (LOAEL) and 0.029 μg/kg (NOAEL), respectively. Yet, Mably et al. (1991, 1992a,b,c) found that a single TCDD dose of 0.064 μg/kg given on day 15 of gestation produces a number of statistically significant effects on the reproductive system of male rat offspring. Because 0.064 μg TCDD/kg was the lowest

dose tested, a NOAEL for developmental male reproductive toxicity, which is defined as the lowest dose used that has no statistically significant effect, could not be determined by Mably et al. (1991, 1992a,b,c). It is concluded that developmental effects on spermatogenesis occur at a maternal TCDD dose that is lower than any previously shown to produce toxicity in rats.

5.4. REFERENCES

Aafjes, J. H.; Vels, J. M.; Schenck, E. (1980) Fertility of rats with artificial oligozoospermia. J. Reprod. Fert. 58: 345-351.

Abbott, B. D.; Birnbaum, L. S. (1989) TCDD alters medial epithelial cell differentiation during palatogenesis. Toxicol. Appl. Pharmacol. 99: 276-286.

Abbott, B. D.; Birnbaum, L. S. (1990a) Rat embryonic palatal shelves respond to TCDD in organ culture. Toxicol. Appl. Pharmacol. 103: 441-451.

Abbott, B. D.; Birnbaum, L. S. (1990b) TCDD-induced altered expression of growth factors may have a role in producing cleft palate and enhancing the incidence of clefts after coadministration of retinoic acid and TCDD. Toxicol. Appl. Pharmacol. 106: 418-432.

Abbott, B. D.; Birnbaum, L. S. (1990c) Effects of TCDD on embryonic ureteric epithelial EGF receptor expression and cell proliferation. Teratology 41: 71-84.

Abbott, B. D.; Birnbaum, L. S. (1991) TCDD exposure of human embryonic palatal shelves in organ culture alters the differentiation of medial epithelial cells. Teratology 43: 119-132.

Abbott, B. D.; Birnbaum, L. S.; Pratt, R. M. (1987a) TCDD-induced hyperplasia of the ureteral epithelium produces hydronephrosis in murine fetuses. Teratology 35: 329-334.

Abbott, B. D.; Morgan, K. S.; Birnbaum, L. S.; Pratt, R. M. (1987b) TCDD alters the extracellular matrix and basal lamina of the fetal mouse kidney. Teratology 35: 335-344.

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

Agrawal, A. K.; Tilson, H. A.; Bondy, S. C. (1981) 3,4,3′,4′-Tetrachlorobiphenyl given to mice prenatally produces long-term decreases in striatal dopamine and receptor binding sites in the caudate nucleus. Toxicol. Lett. 7: 417-424.

Allen, J. R.; Carstens, L. A. (1967) Light and electron microscopic observations in *Macaca mulatta* monkeys fed toxic fat. Am. J. Vet. Res. 28: 1513-1526.

Allen, J. R.; Lalich, J. J. (1962) Response of chickens to prolonged feeding of crude "toxic fat." Proc. Soc. Exp. Biol. Med. 109: 48-51.

Allen, J. R.; Barsotti, D. A.; Van Miller, J. P.; Abrahamson, L. J.; Lalich, J. J. (1977) Morphological changes in monkeys consuming a diet containing low levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Food Cosmet. Toxicol. 15: 401-410.

Allen, J. R.; Barsotti, D. A.; Lambrecht, L. K.; Van Miller, J. P. (1979) Reproductive effects of halogenated aromatic hydrocarbons on nonhuman primates. Ann. N Y Acad. Sci. 320: 419-425.

Allred, P. M.; Strange, J. R. (1977) The effects of 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on developing chicken embryos. Arch. Environ. Contam. Toxicol. 6: 483-489.

Amann, R. P. (1982) Use of animal models for detecting specific alterations in reproduction. Fundam. Appl. Toxicol. 2: 13-26.

Amann, R. P. (1986) Detection of alterations in testicular and epididymal function in laboratory animals. Environ. Health Perspect. 70: 149-158.

Astroff, B.; Safe, S. (1990) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin as an antiestrogen: effect on rat uterine peroxidase activity. Biochem. Pharmacol. 39: 485-488.

Astroff, B.; Rowlands, C.; Dickerson, R.; Safe, S. (1990) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin inhibition of 17β-estradiol-induced increases in rat uterine epidermal growth factor receptor binding activity and gene expression. Mol. Cell. Endocrinol. 72: 247-252.

Aubert, M. L.; Begeot, M.; Winiger, B. P.; Morel, G.; Sizonenko, P. C.; Dubois, P. M. (1985) Ontogeny of hypothalamic luteinizing hormone-releasing hormone (GnRH) and pituitary GnRH receptors in fetal and neonatal rats. Endocrinology 116: 1565-1576.

Balinsky, B. I. (1970) An introduction to embryology. Philadelphia, PA: W.B. Saunders Company; pp. 367-423.

Bardin, C. W.; Cheng, C. Y.; Mustow, N. A.; Gunsalus, G. L. (1988) The Sertoli cell. In: Knobil, E.; Neill, J. D., eds. The physiology of reproduction. New York, NY: Raven Press; pp. 933-974.

Barraclough, C. A. (1980) Sex differentiation of cyclic gonadotropin secretion. In: Kaye, A. M.; Kaye, M., eds. Advances in the biosciences: v. 25. New York, NY: Pergamon Press; pp. 433-450.

Barsotti, D. A.; Abrahamson, L. J.; Allen, J. R. (1979) Hormonal alterations in female rhesus monkeys fed a diet containing 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Bull. Environ. Contam. Toxicol. 21: 463-469.

Biegel, L.; Safe, S. (1990) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on cell growth and the secretion of the estrogen-induced 34-, 52-, and 160-kDa proteins in human breast cancer cells. J. Steroid Biochem. Molec. Biol. 37: 725-732.

Biegel, L.; Harris, M.; Davis, D.; Rosengren, R.; Safe, L.; Safe, S. (1989) 2,2'4,4'5,5'-Hexachlorobiphenyl as a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin antagonist in C57BL/6 mice. Toxicol. Appl. Pharmacol. 97: 561-571.

Binder, B.; Lech, J. J. (1984) Xenobiotics in gametes of Lake Michigan lake trout *Salvelinus namaycush* induce hepatic monooxygenase activity in their offspring. Fundam. Appl. Toxicol. 4: 1042-1054.

Binder, B.; Stegeman, J. J. (1983) Basal levels and induction of hepatic aryl hydrocarbon hydroxylase activity during the embryonic period of development in brook trout. Biochem. Pharmacol. 32: 1324-1327.

Birnbaum, L. S. (1991) Developmental toxicity of TCDD and related compounds: species sensitivities and differences. In: Gallo, M. A.; Scheuplein R. J.; van der Heijden, C. A., eds. Biological basis for risk assessment dioxins and related compounds, Banbury Report 35. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; pp. 51-68.

Birnbaum, L. S.; Weber, H.; Harris, M. W.; Lamb IV, J. C.; McKinney, J. D. (1985) Toxic interaction of specific polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: Increased incidence of cleft palate in mice. Toxicol. Appl. Pharmacol. 77: 292-302.

Birnbaum, L. S.; Harris, M. W.; Miller, C. P.; Pratt, R. M.; Lamb, J. C. (1986) Synergistic interaction of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and hydrocortisone in the induction of cleft palate in mice. Teratology 33: 29-35.

Birnbaum, L. S.; Harris, M. W.; Barnhart, E. R.; Morrissey, R. E. (1987a) Teratogenicity of three polychlorinated dibenzofurans in C57BL/6N mice. Toxicol. Appl. Pharmacol. 90: 206-216.

Birnbaum, L. S.; Harris, M. W.; Crawford, D. D.; Morrissey, R. E. (1987b) Teratogenic effects of polychlorinated dibenzofurans in combination in C57BL/6N mice. Toxicol. Appl. Pharmacol. 91: 246-255.

Birnbaum, L. S.; Harris, M. W.; Stocking, L. M.; Clark, A. M.; Morrissey, R. E. (1989) Retinoic acid and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) selectively enhance teratogenesis in C57BL/6N mice. Toxicol. Appl. Pharmacol. 98: 487-500.

Birnbaum, L. S.; Morrissey, R. E.; Harris, M. W. (1991) Teratogenic effects of 2,3,7,8-tetrabromodibenzo-*p*-dioxin and three polybrominated dibenzofurans in C57BL/6N mice. Toxicol. Appl. Pharmacol. 107: 141-152.

Blazek, J. W.; Ernst, T. L.; Stevens, B. E. (1985) Potential indicators of reproductive toxicity: Testicular sperm production and epididymal sperm number, transit time and motility in Fischer 344 rats. Fundam. Appl. Toxicol. 5: 1097-1103.

Bookstaff, R. C.; Moore, R. W.; Peterson, R. E. (1990a) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin increases the potency of androgens and estrogens as feedback inhibitors of luteinizing hormone secretion in male rats. Toxicol. Appl. Pharmacol. 104: 212-224.

Bookstaff, R. C.; Kamel, F.; Moore, R. W.; Bjęrke, D. L.; Peterson, R. E. (1990b) Altered regulation of pituitary gonadotropin-releasing hormone (GnRH) receptor number and pituitary responsiveness to GnRH in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated male rats. Toxicol. Appl. Pharmacol. 105: 78-92.

Bowman, R. E.; Schantz, S. L.; Gross, M. L.; Ferguson, S. A. (1989a) Behavioral effects in monkeys exposed to 2,3,7,8-TCDD transmitted maternally during gestation and for four months of nursing. Chemosphere 18: 235-242.

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

Brunstrom, B. (1988) Sensitivity of embryos from duck, goose, herring gull, and various chicken breeds to 3,3′,4,4′-tetrachlorobiphenyl. Poult. Sci. 67: 52-57.

Brunstrom, B. (1989) Toxicity of coplanar polychlorinated biphenyls in avian embryos. Chemosphere 19: 765-768.

Brunstrom, B.; Andersson, L. (1988) Toxicity and 7-ethoxyresorufin *O*-deethylase-inducing potency of coplanar polychlorinated biphenyls in chick embryos. Arch. Toxicol. 62: 263-266.

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

Brunstrom, B.; Lund, J. (1988) Differences between chick and turkey embryos in sensitivity to 3,3′,4,4′-tetrachlorobiphenyl and in concentration affinity of the hepatic receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Comp. Biochem. Physiol. 91C: 507-512.

Brunstrom, B.; Reutergardh, L. (1986) Difference in sensitivity of some avian species to the embryotoxicity of a PCB, 3,3′,4,4′-tetrachlorobiphenyl, injected into the eggs. Environ. Pollut. (A) 42: 37-45.

Carlstedt-Duke, J. B. (1979) Tissue distribution of the receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. Cancer Res. 39: 3172-3176.

Chahoud, I.; Krowke, R.; Schimmel, A.; Merker, H. D.; Neubert, D. (1989) Reproductive toxicity and pharmacokinetics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. I. Effects of high doses on the fertility of male rats. Arch. Toxicol. 63: 432-439.

Chen, S.-W.; Roman, B. L.; Saroya, S. Z.; Shinomiya, K.; Moore, R. W.; Peterson, R. E. (1993) In utero exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) does not impair testosterone production by fetal rat testis. The Toxicologist 13: 104.

Cheung, M. O.; Gilbert, E. F.; Peterson, R. E. (1981a) Cardiovascular teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the chick embryo. Toxicol. Appl. Pharmacol. 61: 197-204.

Cheung, M. O.; Gilbert, E. F.; Peterson, R. E. (1981b) Cardiovascular teratogenesis in chick embryos treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. In: Khan, M. A. Q.; Stanton, R. H., eds. Toxicology of halogenated hydrocarbons: health and ecological effects. Elmsford, NY: Pergamon Press; pp. 202-208.

Chou, S. M.; Miike, T.; Payne, W. M.; Davis, G. L. (1979) Neuropathology of "spinning syndrome" induced by prenatal intoxication with a PCB in mice. Ann. NY Acad. Sci. 320: 373-395.

Chung, L. W. K.; Ferland-Raymond, G. (1975) Differences among rat sex accessory glands in their neonatal androgen dependency. Endocrinology 97: 145-153.

Chung, L. W. K.; Raymond, G. (1976) Neonatal imprinting of the accessory glands and hepatic monooxygenases in adulthood. Fed. Proc. 35: 686.

Coffey, D. S. (1988) Androgen action and the sex accessory tissues. In: Knobil, E.; Neill, J., eds. The physiology of reproduction. New York: Raven Press; pp. 1081-1119.

Coleman, R. D. (1965) Development of the rat palate. Anat. Rec. 151: 107-118.

Cook, P. M.; Walker, M. K.; Kuehl, D. W.; Peterson, R. E. (1991) Bioaccumulation and toxicity of TCDD and related compounds in aquatic ecosystems. In: Gallo, M. A.; Scheuplein, R. J.; van der Heijden, C. A., eds. Biological basis for risk assessment of dioxins and related compounds, Banbury Report 35. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; pp. 143-168.

Cooke, B. A.; Platts, E. A.; Abayasekera, R.; Kurlak, L. O.; Schulster, D.; Sullivan, M. H. F. (1989) Control of multiple transducing systems by LH which results in modulation of adenylate cyclase, protein kinase C, lipoxygenases and cyclooxygenases. J. Reprod. Fertil. Suppl. 37: 139-141.

Cooper, K. R. (1989) The effects of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans on aquatic organisms. CRC Crit. Rev. Aquat. Sci. 1: 227-242.

Courtney, K. D. (1976) Mouse teratology studies with chlorodibenzo-*p*-dioxins. Bull. Environ. Contam. Toxicol. 16: 674-681.

Courtney, K. D.; Moore, J. A. (1971) Teratology studies with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. 20: 396-403.

Couture, L. A.; Harris, M. W.; Birnbaum, L. S. (1989) Developmental toxicity of 2,3,4,7,8-pentachlorodibenzofuran in the Fischer 344 rat. Fundam. Appl. Toxicol. 12: 358-366.

Couture, L. A.; Abbott, B. D.; Birnbaum, L. S. (1990a) A critical review of the developmental toxicity and teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: recent advances toward understanding the mechanism. Teratology 42: 619-627.

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

D'Argy, R.; Hassoun, E.; Dencker, L. (1984) Teratogenicity of TCDD and congener 3,3′,4,4′-tetrachloroazoxybenzene in sensitive and nonsensitive mouse strains after reciprocal blastocyst transfer. Toxicol. Lett. 21: 197-202.

Demassa, D. A.; Smith, E. R.; Tennent, B.; Davidson, J. M. (1977) The relationship between circulating testosterone levels and male sexual behavior in rats. Horm. Behav. 8: 275-286.

Dencker, L.; Pratt, R. M. (1981) Association between the presence of the Ah receptor in embryonic murine tissues and sensitivity to TCDD-induced cleft palate. Teratogen. Carcinogen. Mutagen. 1: 399-406.

Denison, M. S.; Okey, A. B.; Hamilton, J. W.; Bloom, S. E.; Wilkinson, C. F. (1986) Ah receptor for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: ontogeny in chick embryo liver. J. Biochem. Toxicol. 1: 39-49.

Desjardins, C.; Jones, R. A. (1970) Differential sensitivity of rat accessory-sex-tissues to androgen following neonatal castration or androgen treatment. Anat. Rec. 166: 299.

DeVito, M. J.; Thomas, T.; Martin, E.; Umbreit, T. H.; Gallo, M. A. (1992) Antiestrogenic action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: tissue-specific regulation of estrogen receptor in CD1 mice. Toxicol. Appl. Pharmacol. 113: 284-292.

Dhar, J. D.; Setty, B. S. (1990) Changes in testis, epididymis and other accessory organs of male rats treated with Anandron during sexual maturation. Endocr. Res. 16: 231-239.

Ehrhardt, A. A.; Meyer-Bahlburg, F. L. (1981) Effects of prenatal sex hormones on gender-related behavior. Science 211: 1312-1317.

Elliott, J. E.; Butler, R. W.; Norstrom, R. J.; Whitehead, P. E. (1989) Environmental contaminants and reproductive success of Great Blue Herons *Ardea herodias* in British Columbia, 1986-87. Environ. Pollut. 59: 91-114.

Eriksson, P. (1988) Effects of 3,3′,4,4′-tetrachlorobiphenyl in the brain of the neonatal mouse. Toxicology 49: 43-48.

Eriksson, P.; Lundkvist, U.; Fredriksson, A. (1991) Neonatal exposure to 3,3′,4,4′-tetrachlorobiphenyl: changes in spontaneous behavior and cholinergic muscarinic receptors in the adult mouse. Toxicology 69: 27-34.

Fara, G. M.; Del Corno, G. (1985) Pregnancy outcome in the Seveso area after TCDD contamination. In: Marois, M., ed. Prevention of physical and mental congenital defects, part b: epidemiology, early detection and therapy, and environmental factors. New York, NY: Alan R. Liss, Inc. pp. 279-285.

Fitchett, J. E.; Hay, E. D. (1989) Medial edge epithelium transforms to mesenchyme after embryonic palatal shelves fuse. Dev. Biol. 131: 455-474.

Forsberg, G.; Abrahamsson, K.; Södersten, P.; Eneroth, P. (1985) Effects of restricted maternal contact in neonatal rats on sexual behavior in the adult. J. Endocrinol. 104: 427-431.

Funatsu, I.; Yamashita, F.; Yosikane, T.; Funatsu, T.; Ito, Y.; Tsugawa, S. (1971) A chlorobiphenyl induced fetopathy. Fukuoka Acta Med. 62: 139-149.

Gallo, M. A.; Hesse, E. J.; McDonald, G. J.; Umbreit, T. H. (1986) Interactive effects of estradiol and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on hepatic cytochrome P-450 and mouse uterus. Toxicol. Lett. 32: 123-132.

Gasiewicz, T. A. (1983) Receptors for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: their inter- and intra-species distribution and relationship to the toxicity of this compound. In: Proceedings of the thirteenth annual conference on environmental toxicology. AFAMRL-TR-82-101, Air Force Aerospace Medical Research Laboratory, Wright-Patterson A.F.B., Ohio; pp. 250-269.

Gasiewicz, T. A.; Giger, L. E.; Rucci, G.; Neal, R. A. (1983) Distribution, excretion, and metabolism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in C57BL/6J, DBA/2J and B6D2F1/J mice. Drug Metab. Dispos. 11: 397-403.

Ghafoorunissa. (1980) Undernutrition and fertility of male rats. J. Reprod. Fertil. 59: 317-320.

Giavini, E. M.; Prati, M.; Vismara, C. (1982a) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin administered to pregnant rats during the preimplantation period. Environ. Res. 29: 185-189.

Giavini, E. M.; Prati, M.; Vismara, C. (1982b) Rabbit teratology studies with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Environ. Res. 27: 74-78.

Giavini, E. M.; Prati, M.; Vismara, C. (1983) Embryotoxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin administered to female rats before mating. Environ. Res. 31: 105-110.

Gierthy, J. F.; Lincoln II, D. W. (1988) Inhibition of postconfluent focus production in cultures of MCF-7 human breast cancer cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Breast Cancer Res. Treat. 12: 227-233.

Gierthy, J. F.; Lincoln II, D. W.; Gillespie, M. B.; Seeger, J. I.; Martinez, H. L.; Dickerman, H. W.; Kumar, S. A. (1987) Suppression of estrogen-regulated extracellular tissue plasminogen activator activity of MCF-7 cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Cancer Res. 47: 6198-6203.

Gilbertson, M. (1989) Effects on fish and wildlife populations. In: Kimbrough, R. D.; Jensen, A. A., eds. Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. 2nd ed. Amsterdam: Elsevier Science Publishers; pp. 103-127.

Glass, A. R.; Herbert, D. C.; Anderson, J. (1986) Fertility onset, spermatogenesis, and pubertal development in male rats: effect of graded underfeeding. Pediatr. Res. 20: 1161-1167.

Gogan, F.; Beattie, I.; Hery, M.; Laplante, E.; Kordon, C. (1980) Effect of neonatal administration of steroids or gonadectomy upon oestradiol-induced luteinizing hormone release in rats of both sexes. J. Endocrinol. 85: 69-74.

Gogan, F.; Slama, A.; Bizzini-Koutznetzova, B.; Dray, F.; Kordon, C. (1981) Importance of perinatal testosterone in sexual differentiation in the male rat. J. Endocrinol. 91: 75-79.

Gorski, R. A. (1974) The neuroendocrine regulation of sexual behavior. In: Newton, G.; Riesen, A. H., eds. Advances in psychobiology: v. 2, New York, NY: John Wiley and Sons; pp. 1-58.

Gorski, R. A.; Gordon, J. H.; Shryne, J. E.; Southam, A. M. (1978) Evidence for a morphological sex difference within the medial preoptic area of the rat brain. Brain Res. 148: 333-346.

Goy, R. W.; Bercovitch, F. B.; McBrair, M. C. (1988) Behavior masculinization is independent of genital masculinization in prenatally androgenized female rhesus macaques. Horm. Behav. 22: 552-571.

Gray, L. E.; Ostby, J. S.; Kelce, W.; Marshall, R.; Diliberto, J. J.; Birnbaum, L. S. (1993) Perinatal TCDD exposure alters sex differentiation in both female and male LE Hooded rats. Abstracts: Dioxin '93, 13th International Symposium on Chlorinated Dioxins and Related Compounds, Vienna, pp. 337-339.

Greene, R. M.; Pratt, R. M. (1976) Developmental aspects of secondary palate formation. J. Embryol. Exp. Morph. 36: 225-245.

Greig, J. B.; Jones, G.; Butler, W. H.; Barnes, J. M. (1973) Toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Food Cosmet. Toxicol. 11: 585-595.

Haake, J. M.; Safe, S.; Mayura, K.; Phillips, T. D. (1987) Arochlor 1254 as an antagonist of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Lett. 38: 299-306.

Hall, P. F. (1988) Testicular steroid synthesis: organization and regulation. In: Knobil, E.; Neill, J. D.; Ewing, L. L.; Greenwald, G. S.; Markert, C. L.; Pfaff, D. W., eds. The physiology of reproduction. New York, NY: Raven Press; pp. 975-998.

Hardy, D. F.; DeBold, J. F. (1972) Effects of coital stimulation upon behavior of the female rat. J. Comp. Physiol. Psychol. 78: 400-408.

Harper, P. A.; Golas, C. L.; Okey, A. B. (1991) Ah receptor in mice genetically "nonresponsive" for cytochrome P450 1A1 induction: cytosolic Ah receptor, transformation to the nuclear binding state, and induction of aryl hydrocarbon hydroxylase by halogenated and nonhalogenated aromatic hydrocarbons in embryonic tissues and cells. Mol. Pharmacol. 40: 818-826.

Harris, M.; Piskorska-Pliszczynska, J.; Romkes, M.; Safe, S. (1989) Structure-dependent induction of aryl hydrocarbon hydroxylase in human breast cancer cell lines and characterization of the Ah receptor. Cancer Res. 49: 4531-4535.

Harris, M.; Zacharewski, T.; Safe, S. (1990) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related compounds on the occupied nuclear estrogen receptor in MCF-7 human breast cancer cells. Cancer Res. 50: 3579-3584.

Hart, B. L. (1972) Manipulation of neonatal androgen: effects on sexual responses and penile development in male rats. Physiol. Behav. 8: 841-845.

Hassoun, E.; d'Argy, R.; Dencker, L.; Lundin, L.-G.; Borwell, P. (1984a) Teratogenicity of 2,3,7,8-tetrachlorodibenzofuran in BXD recombinant inbred strains. Toxicol. Lett. 23: 37-42.

Hassoun, E.; d'Argy, R.; Dencker, L.; Sundstrom, G. (1984b) Teratological studies on the TCDD congener 3,3′,4,4′-tetrachloro-azoxybenzene in sensitive and nonsensitive mouse strains: Evidence for direct effect on embryonic tissues. Arch. Toxicol. 55: 20-26.

Heilmann, L. J.; Sheen, Y.-Y.; Bigelow, S. W.; Nebert, D. W. (1988) Trout P450IA1: cDNA and deduce protein sequence, expression in liver, and evolutionary significance. DNA 7: 379-387.

Helder, T. (1980) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on early life stages of the pike (*Esox lucius* L.) Sci. Total Environ. 14: 255-264.

Helder, T. (1981) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on early life stages of rainbow trout (*Salmo gairdneri*, Richardson). Toxicology 19: 101-112.

Henck, J. M.; New, M. A.; Kociba, R. J.; Rao, K. S. (1981) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: acute oral toxicity in hamsters. Toxicol. Appl. Pharmacol. 59: 405-407.

Hines, M. (1982) Prenatal gonadal hormones and sex differences in human behavior. Psychol. Bull. 92: 56-80.

Hoffman, R. E.; Stehr-Green, P. A. (1989) Localized contamination with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: the Missouri episode. In: Kimbrough, R. D.; Jensen, A. A., eds. Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. 2nd ed. Amsterdam: Elsevier Science Publishers; pp. 471-484.

Hsu, S. T.; Ma, C. I.; Hsu, S. K. H.; Wu, S. S.; Hsu, N. H. M.; Yeh, C. C.; Wu, S. B. (1985) Discovery and epidemiology of PCB poisoning in Taiwan: a four-year followup. Environ. Health Perspect. 59: 5-10.

Israel, D. I.; Whitlock, J. P. (1983) Induction of mRNA specific for cytochrome P_1-450 in wild type and variant mouse hepatoma cells. J. Biol. Chem. 258: 10390-10394.

Jean-Faucher, C.; Berger, M.; Turckheim, M.; Veyssiere, G.; Jean, C. (1982a) The effect of preweaning undernutrition upon the sexual development of male mice. Biol. Neonate 41: 45-51.

Jean-Faucher, C.; Berger, M.; Turckheim, M.; Veyssiere G.; Jean, C. (1982b) Effect of preweaning undernutrition on testicular development in male mice. Int. J. Androl. 5: 627-635.

Jones, K. G.; Sweeney, G. D. (1980) Dependence of the porphyrogenic effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin upon inheritance of aryl hydrocarbon hydroxylase responsiveness. Toxicol. Appl. Pharmacol. 53: 42-49.

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

Khera, K. S. (1992) Extraembryonic tissue changes induced by 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin and 2,3,4,7,8-pentachlorodibenzofuran with a note on direction of maternal blood flow in the labyrinth of C57BL/6N mice. Teratology 45: 611-627.

Khera, K. S.; Ruddick, J. A. (1973) Polychlorodibenzo-p-dioxins: perinatal effects and the dominant lethal test in Wistar rats. In: Blair, E. H., ed. Chlorodioxins-origin and fate. Washington DC: American Chemical Society; pp. 70-84.

Kimmel, G. L. (1988) Appendix C, in the appendices to "A cancer risk-specific dose estimate for 2,3,7,8-TCDD," U.S. EPA, External Review Draft.

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

Kleeman, J. M.; Moore, R. W.; Peterson, R. E. (1990) Inhibition of testicular steroidogenesis in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated rats: evidence that the key lesion occurs prior to or during pregnenolone formation. Toxicol. Appl. Pharmacol. 106: 112-125.

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

Kociba, R. J.; Keyes, D. G.; Beyer, J. E.; Carreon, R. M.; Wade, C. E.; Dittenber, D. A.; Kalnine, R. P.; Frauson, L. E.; Park, C. N.; Barnard, S. D.; Hummel, R. A.; Humiston, C. G. (1978) Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Toxicol. Appl. Pharmacol. 46: 279-303.

Kubiak, T. J.; Harris, H. J.; Smith, L. M.; Schwartz, T. R.; Stalling, D. L.; Trick, J. A.; Sileo, L.; Docherty, D. E.; Erdman, T.C. (1989) Microcontaminants and reproductive impairment of the Forster's tern on Green Bay, Lake Michigan--1983. Arch. Environ. Contam. Toxicol. 18: 706-727.

Kuratsune, M. (1989) Yusho, with reference to Yu-Cheng. In: Kimbrough, R. D.; Jensen, A. A., eds. Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. 2nd ed. Amsterdam: Elsevier Science Publishers; pp. 381-400.

Lambert, G. H.; Nebert, D. W. (1977) Genetically mediated induction of drug-metabolizing enzymes associated with congenital defects in the mouse. Teratology 16: 147-154.

Lambrecht, R. W.; Sinclair, P. R.; Bement, W. J.; Sinclair, J. F. (1988) Uroporphyrin accumulation in cultured chick embryo hepatocytes: comparison of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 3,4,3′4′-tetrachlorobiphenyl. Toxicol. Appl. Pharmacol. 96: 507-516.

Lan, S-J.; Yen, Y-Y.; Ko, Y-C.; Chin, E-R. (1989) Growth and development of permanent teeth germ of transplacental Yu-Cheng babies in Taiwan. Bull. Environ. Contam. Toxicol. 42: 931-934.

Law, K. L.; Hwang, B. T.; Shaio, I. S. (1981) PCB poisoning in newborn twins. Clin. Med. (Taipei) 7: 88-91 (in Chinese).

LeVay, S. (1991) A difference in hypothalamic structure between heterosexual and homosexual men. Science 253: 1034-1037.

Lin, F. H.; Stohs, S. J.; Birnbaum, L. S.; Clark, G.; Lucier, G. W.; Goldstein, J. A. (1991) The effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the hepatic estrogen and glucocorticoid receptors in congenic strains of Ah responsive and Ah nonresponsive C57BL/6 mice. Toxicol. Appl. Pharmacol. 108: 129-139.

Lorenzen, A.; Okey, A. B. (1990) Detection and characterization of [^{3}H]2,3,7,8-tetrachlorodibenzo-*p*-dioxin binding to Ah receptor in a rainbow trout hepatoma cell line. Toxicol. Appl. Pharmacol. 106: 53-62.

Mably, T. A.; Moore, R. W.; Bjerke, D. L.; Peterson, R. E. (1991) The male reproductive system is highly sensitive to in utero and lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin exposure. In: Gallo, M. A.; Scheuplein, R. J.; van der Heijden, C. A., eds. Biological basis for risk assessment of dioxins and related compounds, Banbury Report 35. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; pp. 69-78.

Mably, T. A.; Moore, R. W.; Peterson, R. E. (1992a) In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin: 1. Effects on androgenic status. Toxicol. Appl. Pharmacol. 114: 97-107.

Mably, T. A.; Moore, R. W.; Goy, R. W.; Peterson, R. E. (1992b) In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin: 2. Effects on sexual behavior and the regulation of luteinizing hormone secretion in adulthood. Toxicol. Appl. Pharmacol. 114: 108-117.

Mably, T. A.; Bjerke, D. L.; Moore, R. W.; Gendron-Fitzpatrick, A.; Peterson, R. E. (1992c) In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin: 3. Effects on spermatogenesis and reproductive capability. Toxicol. Appl. Pharmacol. 114: 118-126.

Mac, M. J.; Schwartz, T. R.; Edsall, C. C. (1988) Correlating PCB effects on fish reproduction using dioxin equivalents. Soc. Environ. Toxicol. Chem. Ninth Annu. Meet. Abstr. p. 116.

MacLusky, N. J.; Naftolin, F. (1981) Sexual differentiation of the central nervous system. Science 211: 1294-1303.

Madhukar, B. V.; Brewster, D. W.; Matsumura, F. (1984) Effects of in vivo-administered 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on receptor binding of epidermal growth factor in the hepatic plasma membrane of rat, guinea pig, mouse, and hamster. Proc. Natl. Acad. Sci. (USA) 81: 7407-7411.

Marks, G. S. (1985) Exposure to toxic agents: the heme biosynthetic pathway and hemoproteins as indicator. Crit. Rev. Toxicol. 15: 151-179.

Marks, T. A.; Staples, R. E. (1980) Teratogenic evaluation of the symmetrical isomers of hexachlorobiphenyl (HCB) in the mouse. In: Proceedings 20th Annual Meeting of the Teratology Society, Portsmouth, NH, June 1980. p. 54A.

Marks, T. A.; Kimmel, G. L.; Staples, R. E. (1981) Influence of symmetrical polychlorinated biphenyl isomers on embryo and fetal development in mice. Toxicol. Appl. Pharmacol. 61: 269-276.

Marks, T. A.; Kimmel, G. L.; Staples, R. E. (1989) Influence of symmetrical polychlorinated biphenyl isomers on embryo and fetal development in mice, II. Comparison of 4,4′-dichlorobiphenyl, 3,3′,4,4′-tetrachlorobiphenyl, and 3,3′4,4′-tetramethylbiphenyl. Fundam. Appl. Toxicol. 13: 681-693.

Martin, S.; Duncan, J.; Thiel, D.; Peterson R.; Lemke, M. (1989) Evaluation of the effects of dioxin-contaminated sludges on eastern bluebirds and tree swallows. Report prepared for Nekoosa Papers, Inc., Port Edwards, WI, USA.

Mastroiacova, P.; Spagnolo, A.; Marni, E.; Meazza, L.; Bertollini R.; Segni, G. (1988) Birth defects in the Seveso area after TCDD contamination. JAMA 259: 1668-1672.

McConnell, E. E.; Moore, J. A. (1979) Toxicopathology characteristics of halogenated aromatic hydrocarbons. Ann. NY Acad. Sci. 320: 138-150.

McConnell, E. E.; Moore, J. A.; Haseman, J. K.; Harris, M. W. (1978) The comparative toxicity of chlorinated dibenzo-*p*-dioxins in mice and guinea pigs. Toxicol. Appl. Pharmacol. 44: 335-356.

McEwen, B. S. (1978) Sexual maturation and differentiation: the role of the gonadal steroids. Prog. Brain Res. 48: 281-307.

McEwen, B. S.; Lieberburg, I.; Chaptal, C.; Krey, L. C. (1977) Aromatization: important for sexual differentiation of the neonatal rat brain. Horm. Behav. 9: 249-263.

McNulty, W. P. (1977) Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin for rhesus monkeys: brief report. Bull. Environ. Contam. Tox. 18: 108-109.

McNulty, W. P. (1984) Fetotoxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for rhesus macaques (*Macaca mulatta*). Am. J. Primatol. 6: 41-47.

McNulty, W. P. (1985) Toxicity and fetotoxicity of TCDD, TCDF and PCB isomers in rhesus macaques (*Macaca mulatta*). Environ. Health Perspect. 60: 77-88.

McNulty, W. P.; Nielsen-Smith, K. A.; Lay, J. O., Jr.; Lippstreu, D. L.; Kangas, N. L.; Lyon, P. A.; Gross, M. L. (1982) Persistence of TCDD in monkey adipose tissue. Food Chem. Toxicol. 20: 985-987.

Mebus, C. A.; Reddy, V. R.; Piper, W. N. (1987) Depression of rat testicular 17-hydroxylase and 17,20-lyase after administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Biochem. Pharmacol. 36: 727-731.

Meistrich, M. L. (1992) A method of quantitative assessment of reproductive risks to the human male. Fundam. Appl. Toxicol. 18: 479-90.

Miller, R. W. (1985) Congenital PCB poisoning: a reevaluation. Environ. Health Perspect. 60: 211-214.

Miller, C. P.; Birnbaum, L. S. (1986) Teratologic evaluation of hexabrominated naphthalenes in C57BL/6N mice. Fundam. Appl. Toxicol. 7: 398-405.

Moore, R. W.; Peterson, R. E. (1988) Androgen catabolism and excretion in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated rats. Biochem. Pharmacol. 37: 560-562.

Moore, J. A.; Gupta, B. N.; Zinkl, J. G.; Voss, J. G. (1973) Postnatal effects of maternal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Environ. Health Perspect. 5: 81-85.

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

Moore, J. A.; McConnell, E. E.; Dalgard, D. W.; Harris, M. W. (1979) Comparative toxicity of three halogenated dibenzofurans in guinea pigs, mice, and rhesus monkeys. Ann. NY Acad. Sci. 320: 151-163.

Moore, R. W.; Potter, C. L.; Theobald, H. M.; Robinson, J. A.; Peterson, R. E. (1985) Androgenic deficiency in male rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. 79: 99-111.

Moore, R. W.; Parsons, J. A.; Bookstaff, R. C.; Peterson, R. E. (1989) Plasma concentrations of pituitary hormones in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated male rats. J. Biochem. Toxicol. 4: 165-172.

Moore, R. W.; Jefcoate, C. R.; Peterson, R. E. (1991) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin inhibits steroidogenesis in the rat testis by inhibiting the mobilization of cholesterol to cytochrome $P450_{scc}$. Toxicol. Appl. Pharmacol. 109: 85-97.

Moore, R. W.; Mably, T. A.; Bjerke, D. L.; Peterson, R. E. (1992) In utero and lactational 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) exposure decreases androgenic responsiveness of male sex organs and permanently inhibits spermatogenesis and demasculinizes sexual behavior in rats. Toxicologist 12: 81.

Morrissey, R. E.; Schwetz, B. A. (1989) Reproductive and developmental toxicity in animals. In: Kimbrough, R. D.; Jensen, A. A., eds. Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. 2nd ed. Amsterdam: Elsevier; pp. 195-225.

Morrissey, R. E.; Harris, H. W.; Diliberto, J. J.; Birnbaum, L. S. (1992) Limited PCB antagonism of TCDD-induced malformations in mice. Toxicol. Lett. 60: 19-25.

Murray, F. J.; Smith, F. A.; Nitschke, K. D.; Humiston, C. G.; Kociba, R. J.; Schwetz, B. A. (1979) Three-generation reproduction study of rats given 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the diet. Toxicol. Appl. Pharmacol. 50: 241-252.

Nadler, R. D. (1969) Differentiation of the capacity for male sexual behavior in the rat. Horm. Behav. 1: 53-63.

Nagarkatti, P. S.; Sweeney, G. D.; Gauldie, J.; Clark, D. A. (1984) Sensitivity of suppression of cytotoxic T cell generation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is dependent on the Ah genotype of the murine host. Toxicol. Appl. Pharmacol. 72: 169-176.

Nebert, D. W.; Gielen, J. E. (1972) Genetic regulation of aryl hydrocarbon hydroxylase induction in the mouse. Fed. Proc. 31: 1315-1325.

Neubert, D.; Dillman, I. (1972) Embryotoxic effects in mice treated with 2,4,5-trichlorophenoxy acetic acid and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. N.S. Arch. Pharmacol. 272: 243-264.

Neumann, F.; von Berswordt-Wallrabe, R.; Elger, W.; Steinbeck, H.; Hahn, J. D.; Kramer, M. (1970) Aspects of androgen-dependent events as studied by antiandrogens. Recent Prog. Horm. Res. 26: 337-410.

Nikolaidis, E.; Brunstrom, B.; Dencker, L. (1988a) Effects of the TCDD congeners 3,3′,4,4′-tetrachlorobiphenyl and 3,3′,4,4′-tetrachloroazoxybenzene on lymphoid development in the bursa of Fabricius of the chick embryo. Toxicol. Appl. Pharmacol. 92: 315-323.

Nikolaidis, E.; Brunstrom, B.; Dencker, L. (1988b) Effects of TCDD and its congeners 3,3′,4,4′-tetrachloroazoxybenzene and 3,3′,4,4′-tetrachlorobiphenyl on lymphoid development in the thymus of avian embryos. Pharmacol. Toxicol. 63: 333-336.

Nikolaidis, E.; Brunstrom, B.; Dencker, L.; Veromaa, T. (1990) TCDD inhibits the support of B-cell development by the bursa of Fabricius. Pharmacol. Toxicol. 67: 22-26.

Nosek, J. A.; Sullivan, J. R.; Hurley, S. S.; Craven, S. R.; Peterson, R. E. (1991) Toxicity and reproductive effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in ring-necked pheasant hens. J. Toxicol. Environ. Health 35: 187-198.

Nosek, J. A.; Sullivan, J. R.; Craven, S. R.; Gendron-Fitzpatrick, A.; Peterson, R. E. (1993) Embryotoxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in ring-necked pheasants. Environ. Toxicol. Chem. 12: 1215-1222.

Okey, A. B.; Bondy, G. P.; Mason, M. E.; Kahl, G. F.; Eisen, H. J.; Guenther, T. M.; Nebert, D. W. (1979) Regulatory gene product of the Ah locus. Characterization of the cytosolic inducer-receptor complex and evidence for its nuclear translocation. J. Biol. Chem. 254: 11636-11648.

Okey, A. B.; Vella, L. M.; Harper, P. A. (1989) Detection and characterization of a low-affinity form of cytosolic Ah receptor in livers of mice nonresponsive to induction of cytochrome P_1-450 by 3-methylcholanthrene. Mol. Pharmacol. 35: 823-830.

Olson, J. R.; McGarrigle, B. P. (1990) Characterization of the developmental toxicity of 2,3,7,8-TCDD in the Golden Syrian hamster. Toxicologist 10: 313.

Olson, J. R.; McGarrigle, B. P. (1992) Comparative developmental toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Chemosphere 25: 71-74.

Olson, J. R.; Holscher, M. A.; Neal, R. A. (1980) Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the Golden Syrian hamster. Toxicol. Appl. Pharmacol. 55: 67-78.

Olson, J. R.; McGarrigle, B. P.; Tonucci, D. A.; Schecter, A.; Eichelberger, H. (1990) Developmental toxicity of 2,3,7,8-TCDD in the rat and hamster. Chemosphere 20: 1117-1123.

Orth, J. M.; Gunsalus, G. L.; Lamperti, A. A. (1988) Evidence from Sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. Endocrinology 122: 787-794.

Osborne, R.; Greenlee, W. F. (1985) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) enhances terminal differentiation of cultured human epidermal cells. Toxicol. Appl. Pharmcol. 77: 434-443.

Payne, A. H.; Quinn, P. G.; Stalvey, J. R. D. (1985) The stimulation of steroid biosynthesis by luteinizing hormone. In: Ascoli, M., ed. Luteinizing hormone action and receptors. Boca Raton, FL: CRC Press; pp. 135-172.

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

Poland, A.; Glover, E. (1980) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: segregation of toxicity with the Ah locus. Mol. Pharmacol. 17: 86-94.

Poland, A.; Knutson, J. C. (1982) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. Ann. Rev. Pharmacol. Toxicol. 22: 517-554.

Pomerantz, S. M.; Goy, R. W.; Roy, M. M. (1986) Expression of male-typical behavior in adult female pseudohermaphrodotic rhesus: comparisons with normal males and neonatally gonadectomized males and females. Horm. Behav. 20: 483-500.

Pratt, R. M.; Dencker, L.; Diewert, V. M. (1984) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin-induced cleft palate in the mouse: evidence for alterations in palatial shelf fusion. Teratogen. Carcinogen. Mutagen. 4: 427-436.

Pratt, R. M.; Kim, C. S.; Goulding, E. H.; Willis, W. D.; Russell, M. M.; Grove, R. I. (1985) Mechanisms of environmentally induced cleft palate. In: Marois, M., ed. Prevention of physical and mental congenital defects, part c: basic and medical science, education, and future strategies. New York, NY: Alan R. Liss, Inc.; pp. 283-287.

Quilley, C. P.; Rifkind, A. B. (1986) Prostaglandin release by the chick embryo heart is increased by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and by other cytochrome P-448 inducers. Biochem. Biophys. Res. Comm. 136: 582-589.

Raisman, G.; Field, P. M. (1973) Sexual dimorphism in the neuropil of the preoptic area of the rat and its dependence on neonatal androgen. Brain Res. 54: 1-29.

Rajfer, J.; Coffey, D. S. (1979) Effects of neonatal steroids on male sex tissues. Invest. Urol. 17: 3-8.

Rajfer, J.; Walsh, P. C. (1977) Hormonal regulation of testicular descent: experimental and clinical observations. Urology 118: 985-990.

Reggiani, G. M. (1989) The Seveso accident: medical survey of a TCDD exposure. In: Kimbrough, R. D.; Jensen, A. A., eds. Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. 2nd ed. Amsterdam: Elsevier Science Publishers; pp. 445-470.

Rier, S. E.; Martin, D. C.; Bowman, R. E.; Dmowski, W. P.; Becker, J. L. (1993) Endometriosis in rhesus monkeys (Macaca mulatta) following chronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Fund. Appl. Toxicol. 21: 433-441.

Rifkind, A. B.; Muschick, H. (1983) Benoxaprofen suppression of polychlorinated biphenyl toxicity without alteration of mixed function oxidase function. Nature (London) 303: 524-526.

Rifkind, A. B.; Sassa, S.; Reyes, J.; Muschick, H. (1985) Polychlorinated aromatic hydrocarbon lethality, mixed-function oxidase induction, and uroporphyrinogen decarboxylase inhibition in the chick embryo: dissociation of dose-response relationships. Toxicol. Appl. Pharmacol. 78: 268-279.

Robaire, B.; Hermo, L. (1989) Efferent ducts, epididymis, and vas deferens: structure, functions, and their regulation. In: Knobil, E.; Neill, J. D., eds. The physiology of reproduction. New York: Raven Press; pp. 999-1080.

Robb, G. W.; Amann, R. P.; Killian, G. J. (1978) Daily sperm production and epididymal sperm reserves of pubertal and adult rats. J. Reprod. Fertil. 54: 103-107.

Robinson, J. R.; Considine, N.; Nebert, D. W. (1974) Genetic expression of aryl hydrocarbon hydroxylase induction. Evidence for the involvement of other loci. J. Biol. Chem. 249: 5851-5859.

Rogan, W. J. (1982) PCBs and Cola-colored babies: Japan, 1968 and Taiwan, 1979. Teratology. 26: 259-261.

Rogan, W. J. (1989) Yu-Cheng. In: Kimbrough, R. D.; Jensen, A. A., eds. Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. 2nd ed. Amsterdam: Elsevier Science Publishers; pp. 401-415.

Rogan, W. J.; Gladen, B. C.; Hung, K.-L.; Koong, S.-L.; Shih, L.-Y.; Taylor, J. S.; Wu, Y.-C.; Yang, D. C.; Ragan, N. B.; Hsu, C.-C. (1988) Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 241: 334-338.

Romkes, M.; Safe, S. (1988) Comparative activities of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and progesterone as antiestrogens in the female rat uterus. Toxicol. Appl. Pharmacol. 92: 368-380.

Romkes, M.; Piskorska-Pliszcynska, J.; Safe, S. (1987) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on hepatic and uterine estrogen receptor levels in rats. Toxicol. Appl. Pharmacol. 87: 306-314.

Rose, J. Q.; Ramsey, J. C.; Wentzler, T. H.; Hummel, R. A.; Gehring, P. J. (1976) The fate of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin following single and repeated oral doses to the rat. Toxicol. Appl. Pharmacol. 36: 209-226.

Ruangwises, S.; Bestervelt, L. L.; Piper, D. W.; Nolan, C. J.; Piper, W. N. (1991) Human chorionic gonadotropin treatment prevents depressed 17α-hydroxylase/C_{17-20} lyase activities and serum testosterone concentrations in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin treated rats. Biol. Reprod. 45: 143-150.

Russell, L. D.; Peterson, R. N. (1984) Determination of the elongate spermatid-Sertoli cell ratio in various mammals. J. Reprod. Fertil. 70: 635-641.

Sachs, B. D.; Barfield, R. J. (1976) Functional analysis of masculine copulatory behavior in the rat. Adv. Study Behav. 7: 91-154.

Safe, S. (1990) Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit. Rev. Toxicol. 21: 51-88.

Safe, S.; Astroff, B.; Harris, M.; Zacharewski, T.; Dickerson, R.; Romkes, M.; Biegel, L. (1991) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds as antiestrogens: characterization and mechanism of action. Pharmacol. Toxicol. 69: 400-409.

Sassa, S.; Sugita, O.; Ohnuma, N.; Imajo, S.; Okumura, T.; Noguchi T.; Kappas A. (1986) Studies of the influence of chloro-substituent sites and conformational energy in polychlorinated biphenyls on uroporphyrin formation in chick-embryo liver cell cultures. Biochem. J. 235: 291-296.

Schantz, S. L.; Bowman, R. E. (1989) Learning in monkeys exposed perinatally to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Neurotox. Teratol. 11: 13-19.

Schantz, S. L.; Barsotti, D. A.; Allen, J. R. (1979) Toxicological effects produced in nonhuman primates chronically exposed to fifty parts per trillion 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Toxicol. Appl. Pharmacol. 48(Part 2): A180.

Schantz, S. L.; Laughlin, M. K.; Van Valkenberg, H. C.; Bowman, R .E. (1986) Maternal care by rhesus monkeys of infant monkeys exposed to either lead or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Neurotoxicology 7: 641-654.

Schantz, S. L.; Mably, T. A.; Peterson, R. E. (1991) Effects of perinatal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on spatial learning and memory and locomotor activity in rats. Teratology 43: 497.

Schwetz, B. A.; Norris, J. M.; Sparschu, G. L.; Rowe, V. K.; Gehring, P. J.; Emerson, J. L.; Gerbig, C. G. (1973) Toxicology of chlorinated dibenzo-*p*-dioxins. Environ. Health Perspect. 5: 87-99.

Seefeld, M. D.; Albrecht, R. M.; Peterson, R. E. (1979) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on indocyanine green blood clearance in rhesus monkeys. Toxicology 14: 263-272.

Seefeld, M. S.; Corbett, S. W.; Keesey, R. E.; Peterson, R. E. (1984) Characterization of the wasting syndrome in rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. 73: 311-322.

Seegal, R. F.; Bush, B.; Shain, W. (1990) Lightly chlorinated ortho-substituted PCB congeners decrease dopamine in nonhuman primate brain and in tissue culture. Toxicol. Appl. Pharmacol. 106: 136-144.

Setty, B. S.; Jehan, Q. (1977) Functional maturation of the epididymis in the rat. J. Reprod. Fertil. 49: 317-322.

Shiverick, K. T.; Muther, T. F. (1982) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on serum concentrations and the uterotrophic action of exogenous estrone in rats. Toxicol. Appl. Pharmacol. 65: 170-176.

Shiverick, K. T.; Muther, T. F. (1983) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) effects on hepatic microsomal steroid metabolism and serum estradiol of pregnant rats. Biochem. Pharmacol. 32: 991-995.

Shuler, C. F.; Halpern, D. E.; Guo Y.; Sank, A. C. (1991) Medial edge epithelium (MEE) fate traced by cell linkage analysis during epithelial-mesenchymal transformation in vivo. J. Cell Biol. 115: 147a (abstr).

Shum, S.; Jensen, N. M.; Nebert, D. W. (1979) The murine Ah locus: in utero toxicity and teratogenesis associated with genetic differences in benzo(a)pyrene metabolism. Teratology 20: 365-376.

Silbergeld, E. K. (1992) Dioxin: distribution of Ah receptor binding in neurons and glia from rat and human brain. Toxicologist 12: 196.

Silkworth, J. B.; Cutler, D. S.; Antrim, L.; Houston, D.; Tumasonis, C.; Kaminsky, L. S. (1989) Teratology of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a complex enviromental mixture from the Love Canal. Fundam. Appl. Toxicol. 13: 1-15.

Sinclair, P. R.; Bement, W. J.; Bonkovsky, H. L.; Sinclair, J. F. (1984) Inhibition of uroporphyrinogen decarboxylase by halogenated biphenyls in chick hepatocyte cultures. Biochem. J. 222: 737-748.

Smith, F. A.; Schwetz, B. A.; Nitschke, K. D. (1976) Teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in CF-1 mice. Toxicol. Appl. Pharmacol. 38: 517-523.

Smith, L. M.; Schwartz, T. R.; Feltz, K.; Kubiak, T. J. (1990) Determination and occurrence of AHH-active polychlorinated biphenyls, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorodibenzofuran in Lake Michigan sediment and biota. The question of their relative toxicological significance. Chemosphere 21: 1063-1085.

Södersten, P.; Hansen, S. (1978) Effects of castration and testosterone, dihydrotestosterone or oestradiol replacement treatment in neonatal rats on mounting behavior in the adult. J. Endocrinol. 76: 251-260.

Sparschu, G. L.; Dunn, F. L.; Rowe, V. K. (1971) Study of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. Food Cosmet. Toxicol. 9: 405-412.

Spink, D. C.; Lincoln II, D. W.; Dickerman, H. W.; Gierthy, J. F. (1990) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin causes an extensive alteration of 17β-estradiol metabolism in MCF-7 breast tumor cells. Proc. Natl. Acad. Sci. (USA) 87: 6917-6921.

Spink, D. C.; Eugster, H. P.; Lincoln II, D. W.; Schuetz, J. D.; Schuetz, E. G.; Johnson, J. A.; Kaminsky, L. A.; Gierthy, J. F. (1992) 17β-Estradiol hydroxylation catalyzed by human cytochrome P450 1A1: a comparison of the activities induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in MCF-7 cells with those from heterologous expression of the cDNA. Arch. Biochem. Biophys. 293: 342-348.

Spitsbergen, J. M.; Walker, M. K.; Olson, J. R.; Peterson, R. E. (1991) Pathologic alterations in early life stages of lake trout, *Salvelinus namaycush*, exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as fertilized eggs. Aquatic Toxicol. 19: 41-72.

Stalling, D. L.; Smith, L. M.; Petty, J. D.; Hogan, J. W.; Johnson, J. L.; Rappe, C.; Buser, H. R. (1983) Residues of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in Laurentian Great Lakes fish. In: Tucker, R. E.; Young, A. L.; Gray, A. P., eds. Human and environmental risks of chlorinated dioxins and related compounds. New York, NY: Plenum Press; pp. 221-240.

Steinberger, E.; Steinberger, A. (1989) Hormonal control of spermatogenesis. In: DeGroot, L. J., ed. Endocrinology. 2nd ed. Philadelphia, PA: W.B. Saunders Co.; pp. 2132-2136.

Stockbauer, J. W.; Hoffman, R. E.; Schramm W. F.; Edmonds, L. D. (1988) Reproductive outcomes of mothers with potential exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. J. Epidemiol. 128: 410-419.

Sunahara, G. I.; Nelson, K. G.; Wong, T. K.; Lucier, G. W. (1987) Decreased human birth weights after in utero exposure to PCBs and PCDFs are associated with decreased placental EGF-stimulated receptor autophosphorylation capacity. Mol. Pharmacol. 32: 572-578.

Taki, I.; Hisanaga S.; Amagase, Y. (1969) Report on Yusho (chlorobiphenyls poisoning) pregnant women and their fetuses. Fukuoka Acta Med. 60: 471-474 (Japan).

Tanabe, S. (1988) PCB problems in the future: foresight from current knowledge. Environ. Pollut. 50: 5-28.

Taleisnik, S.; Caligaris, L.; Astrada, J. J. (1969) Sex difference in the release of luteinizing hormone evoked by progesterone. J. Endocrinol. 44: 313-321.

Thiel, D. A.; Martin, S. G.; Duncan, J. W.; Lemke, M. J.; Lance, W. R.; Peterson, R. E. (1988) Evaluation of the effects of dioxin-contaminated sludges on wild birds. TAPPI Proceedings, 1988 Environmental Conference; pp. 487-506.

Thornton, J.; Goy, R. W. (1986) Female-typical sexual behavior of rhesus and defeminization by androgens given prenatally. Horm. Behav. 20: 129-147.

Tilson, H. A.; Davis, G. J.; McLachlan, J. A.; Lucier, G. W. (1979) The effects of polychlorinated biphenyls given prenatally on the neurobehavioral development of mice. Environ. Res. 18: 466-474.

U.S. EPA (1991) Bioaccumulation of selected pollutants in fish: v. 1, a national study. Office of Water Regulations and Standards, Washington, DC. EPA 506/6-90/001a.

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

Van Miller, J. P.; Lalich, J. J.; Allen, J. R. (1977) Increased incidence of neoplasms in rats exposed to low levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Chemosphere 6: 537-544.

Vecchi, A.; Sironi, M.; Antonia, M.; Recchia, C. M.; Garattini, S. (1983) Immunosuppressive effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in strains of mice with different susceptibility. Natl. Acad. Sci. (USA). 87: 6917-6921.

Vos, G. J.; Moore, J. A. (1974) Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Int. Arch. Allergy Appl. Immunol. 47: 777-794.

Walker, M. K.; Peterson, R. E. (1991) Potencies of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyl congeners for producing early life stage mortality in rainbow trout, (*Oncorhyncus mykiss*). Aquatic Toxicol. 21: 219-238.

Walker, M. K.; Spitsbergen, J. M.; Olson, J. R.; Peterson, R. E. (1991) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin toxicity during early life stage development of lake trout (*Salvelinus namaycush*). Can. J. Fish. Aquat. Sci. 48: 875-883.

Walker, M. K.; Hufnagle, L. C., Jr.; Clayton, M. C.; Peterson, R. E. (1992) An egg injection method for assessing early life stage mortality of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in rainbow trout (Oncorhynchus mykiss). Aquat. Toxicol. 22: 15-38.

Warren, D. W.; Haltmeyer, G. C.; Eik-nes, K. B. (1975) The effect of gonadotrophins on the fetal and neonatal rat testis. Endocrinology 96: 1226-1229.

Warren, D. W.; Huhtaniemi, I. T.; Tapanainen, J.; Dufau, M. L.; Catt, K. J. (1984) Ontogeny of gonadotropin receptors in the fetal and neonatal rat testis. Endocrinology 114: 470-476.

Weber, H.; Birnbaum, L. S. (1985) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) in pregnant C57BL/6 mice: distribution to the embryo and excretion. Arch. Toxicol. 57: 159-162.

Weber, H.; Harris, M. W.; Haseman, J. K.; Birnbaum, L. S. (1985) Teratogenic potency of TCDD, TCDF and TCDD-TCDF combinations in C57BL/6N mice. Toxicol. Lett. 26: 159-167.

Whalen, R. E.; Olsen, K. L. (1981) Role of aromatization in sexual differentiation: effects of prenatal ATD treatment and neonatal castration. Horm. Behav. 15: 107-122.

Wilson, J. D.; George, F. W.; Griffin, J. F. (1981) The hormonal control of sexual development. Science 211: 1278-1284.

Wisk, J. D.; Cooper, K. R. (1990a) The stage specific toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in embryos of the Japanese Medaka (*Oryzias latipes*). Environ. Toxicol. Chem. 9: 1159-1169.

Wisk, J.D.; Cooper, K.R. (1990b) Comparison of the toxicity of several polychlorinated dibenzo-*p*-dioxins and 2,3,7,8-tetrachlorodibenzofuran in embryos of the Japanese medaka (*Oryzias latipes*). Chemosphere 20: 361-377.

Wong, K. C.; Hwang, M. Y. (1981) Children born to PCB poisoning mothers. Clin. Med. (Taipai) 7: 83-87 (in Chinese).

Working, P. K. (1988) Male reproductive toxicology: comparison of the human to animal models. Environ. Health Perspect. 77: 37-44.

Working, P. K.; Hurtt, M. E. (1987) Computerized videomicrographic analysis of rat sperm motility. J. Androl. 8: 330-337.

OHEA-I-486f
May 1994
Internal Review Draft

Chapter 6. Carcinogenicity of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) in Animals

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

NOTICE

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

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

DRAFT--DO NOT QUOTE OR CITE

DISCLAIMER

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

6. CARCINOGENICITY OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN IN ANIMALS

6.1. INTRODUCTION

Additional scientific information on the use of animal cancer data for estimating human risks from 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has become available since 1988. Much of the data on tumor incidence in experimental animals available in 1988 demonstrated that TCDD is a carcinogen at multiple sites in both sexes of rats and mice. Some of the cancers occurred following particularly low doses. Since 1988, TCDD has been shown to be a carcinogen in hamsters, and during the past 3 years, some of the tumor incidence data in rat liver have been reevaluated.

In the past few years, there have been several studies that have impact on the evaluation of cancer studies in experimental animals. For example, the evidence is now considerably stronger that TCDD does not damage DNA directly through the formation of DNA adducts. However, mechanisms have been proposed supporting the possibility that TCDD might alter the DNA-damaging potential of some endogenous compounds, including estrogens. In addition, there have been numerous reports on TCDD-mediated modifications of growth factor pathways and cytokines in experimental animals and cell systems. Some of the altered systems include those for epidermal growth factor, transforming growth factor α, estrogen, glucocorticoids, tumor necrosis factor-α, interleukin 1β, plasminogen inactivating factor, and gastrin. Many of these pathways are involved in cell proliferation and differentiation and provide plausible avenues for researching the mechanisms responsible for the carcinogenic actions of TCDD. These effects are consistent with the generally accepted conclusion that TCDD acts as a "tumor promoter" in multistage models for chemical carcinogenesis and is virtually devoid of initiating activity in these models. It is important to note that "tumor promotion" is an operational and not a mechanistic term and that multiple mechanisms of tumor promotion are likely. Each of these mechanisms may be fundamentally different from the other.

Over the past few years, there has been growing consensus that most, if not all, of TCDD's biochemical and toxic effects require interaction with the aryl hydrocarbon (Ah)

receptor. The properties of the Ah receptor and the mechanisms whereby this receptor regulates gene expression will be evaluated in other chapters. However, formation of the Ah receptor-TCDD complex is only the first of many steps involved in the production of a biochemical and toxic effect. Although we are gaining knowledge of details of the subsequent steps, we know very little about some components of the Ah receptor-mediated responses. It is clear, however, that cell-specific factors other than the Ah receptor must be involved in determining tissue responses once TCDD binds the Ah receptor.

Evaluation of dose response is one of the more important issues that affect dioxin risk assessments. The focus of this controversy centers on whether the effects of dioxin exhibit thresholds. It now appears that for some responses there is a proportional relationship between receptor occupancy and response, which is evidenced by a linear relationship between target dose and effect over a wide dose range. However, different dose-response relationships are seen for different responses so it is probably inappropriate to use a single surrogate marker to estimate dioxin's risks. Furthermore, these data reveal there is no unifying dose-response relationship for all Ah receptor-mediated events.

Another controversial area in risk assessment is whether experimental animal models are appropriate for estimating human risks. During the past few years, there has been increasing evidence that biochemical and toxic responses resulting from human exposure to TCDD and its structural analogs appear to be similar to responses in experimental animals. However, it may be possible that humans are sensitive or resistant for some responses. There also is increasing awareness that interindividual variations in human responses to dioxin are a complicating factor in risk assessment; it appears there are individuals who are responsive and nonresponsive to numerous environmental chemicals, including TCDD.

Much of the controversy surrounding dioxin risk assessment reflects the selection of mathematical models: threshold, linear multistage, or others. We now know considerably more about the mechanisms of actions of dioxin, and this knowledge may permit the construction of biologically based models that reduce some of the uncertainty in current risk estimates. These approaches and recent advances on mechanisms of tumor promotion and dose-response relationships for biochemical and biological events relevant to the carcinogenic actions of dioxin will be discussed in more detail in Chapter 8, Dose-Response Models.

6.2. ANIMAL BIOASSAYS FOR CANCER

Several long-term bioassays for carcinogenicity of TCDD have been conducted in several species. All studies have produced positive results. It is clear that TCDD is a multisite carcinogen in both sexes of rats and mice (Huff et al., 1991; Zeise et al., 1990). It also is a carcinogen in the hamster, which is considered the most resistant species to the acute toxic effects of TCDD. The important studies are summarized in Table 6-1, including information on species, sex, and tumor site. The Kociba and National Toxicology Program (NTP) studies are the most comprehensive and relevant to risk characterization and are described in the following paragraphs.

6.2.1. Kociba Study

The most cited cancer bioassay for TCDD was published by Kociba et al. (1978). It was a lifetime feeding study of male and female Sprague-Dawley rats using doses of 0, 1, 10, and 100 ng/kg/day. There were 50 males and 50 females in each group. Data derived from these studies have been used as the basis for many risk assessments for TCDD. The most significant finding was an increase in hepatocellular hyperplastic nodules and hepatocellular carcinomas in female rats. The carcinomas were significantly elevated above the control incidence at the 100 ng/kg/day dose, whereas increased incidences of hyperplastic nodules were evident in the 10 ng/kg/day dose group. There have been two reevaluations of the Kociba slides of liver sections (Squire, 1980; Sauer, 1990). The Squire review was requested by EPA as an independent review of the slides. The Sauer review used diagnostic criteria for liver tumors described by Maronpot et al. (1986). Liver tumor incidences for the three evaluations are compared in Table 6-2. Although there are some quantitative differences in the evaluations, the lowest detectable effect is consistently at 10 ng/kg/day for liver tumor incidence. In the 10 ng/kg/day dose group, hyperplastic nodules of the liver were observed in female rats (18 in Kociba, 27 in Squire). Two females had carcinomas of the liver. In the recent reevaluation of liver lesions by Sauer (1990), nine females were identified with hepatocellular adenomas and none with carcinomas; thus only one-third of the previously observed tumors were confirmed. There was no detectable increase in liver tumor incidences in male rats (Table 6-1) in any of the dose groups. The mechanism responsible

Table 6-1. Sites for Increased Cancer in Animal Bioassays

Species/Strain/Study	Sex	Site
Rats/Sprague-Dawley Kociba et al., 1978	male	tongue nasal turbinates/hard palate
	female	lung nasal turbinates/hard palate liver
Rats/Osborne-Mendel NTP, 1982a	male	thyroid adrenal cortex
	female	liver adrenal cortex subcutaneous fibrosarcoma
Mice/B6C3F1 NTP, 1982a	male	liver
	female	subcutaneous fibrosarcoma liver thyroid
Mice/B6C3 and B6C Della Porta et al., 1987	male	thymic lymphomas
	female	liver
Hamsters/Syrian Golden Rao et al., 1988	male	facial skin carcinoma

Table 6-2. Different Evaluations of Kociba Liver Tumor Data in Female Rats[a,b]

Study	Tumor type[c]	Control	Dose (ng/kg/day)		
			1	10	100
Kociba et al., 1978	hyperplastic nodule	8/86 p<0.0001	3/50 p=0.8	18/50 p<0.001	23/50 p<0.001
	hepatocellular carcinoma	1/86 p<0.0001	0/50 --	2/50 p=0.3	11/50 p<0.001
	hyperplastic nodule; hepatocellular carcinoma	9/86 p<0.0001	3/50 p=0.7	20/50 p<0.001	34/50 p<0.001
Squire, 1980	neoplastic nodule; hepatocellular carcinoma	16/68 p<0.0001	8/50 p=0.7	27/50 p<0.001	33/47 p<0.001
Sauer, 1990	hepatocellular adenoma	2/86 p<0.0001	1/50	9/50 p<0.01	14/50 p<0.001
	hepatocellular carcinoma	0/86 p<0.01	0/50	0/50	4/50 p<0.05
	hepatocellular adenoma; hepatocellular carcinoma	2/86 p<0.0001	1/50 --	9/50 p<0.01	18/50 p<0.001

[a]Source: Kociba et al., 1978.

[b]p-Values for Fisher's exact test are given below the incidence data for TCDD-treated animals; Cochran-Armitage trend test p-values are given below the control incidences.

[c]Hyperplastic nodule, neoplastic nodule, and hepatocellular adenoma are equivalent and interchangeable lesions.

for dioxin-mediated sex specificity for hepatocarcinogenesis in rats is not clear but may involve estrogens. This is discussed in the section on tumor promotion.

Kociba et al. (1978) had reported that chemically related preneoplastic or neoplastic lesions were not found in the 1 ng/kg/day dose group. However, Squire identified two male rats in the 1 ng/kg/day dose group with squamous cell carcinoma of the nasal turbinates/hard palate, and one of these male rats had a squamous cell carcinoma of the tongue. These are both rare tumors for Sprague-Dawley rats, and these sites are targets for TCDD, implying that the 1 ng/kg/day may not represent a no-observed-effect level (NOEL). However, no dose-response relationships were evident for tumors at these sites (Huff et al., 1991).

In addition to carcinoma of the liver, tongue, nasal turbinates, and hard palate, increased lung tumor incidences were observed in female rats (seven in Kociba, nine in Squire). The increase at the high dose (100 ng/kg/day) was statistically significant for keratinizing squamous cell carcinomas.

One of the more interesting findings in the Kociba bioassay was reduced tumor incidences of the pituitary, uterus, mammary gland, pancreas, and adrenals. For example, carcinomas of the mammary gland occurred in 8 of 86 control female rats, whereas the incidence was 0/49 in the 1 ng/kg/day dose group. However, the incidence of mammary gland carcinomas in the medium- and high-dose groups was similar to that of control rats, suggesting that protection against breast cancer might be a low-dose effect. These findings, coupled with the sex specificity of TCDD-induced liver tumors, emphasize that the carcinogenic actions of TCDD involve a complex interaction of hormonal factors. Moreover, it appears likely that cell-specific factors modulate TCDD/hormone actions relevant to cancer. There is considerable controversy concerning the possibility that TCDD-induced liver tumors are a consequence of cytotoxicity. Goodman and Sauer (1992) have extended the reevaluation of the Kociba slides to include liver toxicity data and have reported a correlation between the presence of overt hepatotoxicity and the development of hepatocellular neoplasms in female rats. With the exception of two tumors in controls and one each in the low- and mid-dose groups, all liver tumors occurred in livers showing clear signs of toxicity. However, male rat livers exhibit cytotoxicity in response to high TCDD doses, yet they do not develop liver tumors. Moreover, both intact and ovariectomized

female rats exhibit liver toxicity in response to TCDD, yet TCDD is a promoter in intact but not ovariectomized rats (Lucier et al., 1991). Therefore, it appears that if cytotoxicity is playing a role in liver tumorigenesis, other factors must also be involved. Also, there is little information on the role of cytotoxicity in TCDD-mediated cancer at other sites such as the lung and thyroid.

6.2.2. NTP Study

The NTP study was conducted using Osborne-Mendel rats and B6C3F1 mice (NTP, 1982a). Groups of 50 male rats, 50 female rats, and 50 male mice received doses of 0, 10, 50, or 500 ng/kg/week TCDD by gavage in two administrations each week for 2 years; groups of 50 female mice were given 0, 40, 200, or 2000 ng/kg/week. These exposures correspond to average daily doses of 1.4, 7.1, or 71 ng/kg/day for rats and male mice and to doses of 5.7, 28.6, or 286 ng/kg/day for female mice, so the doses were roughly similar to those used in the Kociba dietary study. There were no statistically significant dose-related decreases in survival in any sex-species group.

Tumor data in the NTP bioassay are summarized in Tables 6-3 and 6-4. TCDD-induced malignant liver tumors occurred in the high-dose female rats and in male and female mice. These can be considered to result from TCDD exposure because they are relatively uncommon lesions in control Osborne-Mendel rats (male, 1/208; female, 3/208), are seen in female rats and mice of both sexes, and their increasing incidence with increasing dose is statistically significant (Cochran-Armitage trend test, $p=0.004$). Because liver tumors were increased in both sexes of mice, this effect is not female specific as observed in rats. Interestingly, liver tumor incidences were decreased in female rats in both the NTP and Kociba low doses (not statistically significant compared with controls). For example, the combined control incidence data were 11/161 (7%) compared with 4/99 (4%) in the low-dose group.

The incidences of thyroid gland (follicular cell) tumors were increased in all three dose groups in male rats. Because the responses in the two highest dose groups are highly significant, the statistically significant elevation of incidence in the lowest dose group (Fisher exact p-value=0.042) is considered to be caused by exposure to TCDD. Thus, for this study

Table 6-3. Tumor Incidences in Male and Female Osborne-Mendel Rats Given TCDD by Gavage for 2 Years[a,b]

Target organ/tumor type	Sex	Dose (ng/kg/day)			
		0	1.4	7.1	71
Thyroid follicular cell adenoma	males	1/69 p=0.006	5/48 p=0.042	6/50 p=0.021	10/50 p=0.001
Liver neoplastic nodule		0/74 p=0.005	0/50 --	0/50 --	3/50 p=0.06
Adrenal cortex adenoma		6/72 p=0.26	9/50 p=0.09	12/49 p=0.015	9/49 p=0.09
Liver neoplastic nodule	females	5/75 p<0.001	1/49 --	3/50 --	12/49 p=0.006
Adrenal cortex adenoma or carcinoma		11/73 p=0.014	9/49 p=0.4	5/49 --	14/46 p=0.039
Subcutaneous fibrosarcoma		0/75 --	2/50 p=0.16	3/50 p=0.06	4/49 p=0.023

[a]Source: NTP, 1982a.

[b]p-Values under the tumor incidence data of controls are from Cochran-Armitage test for dose-related trend, and p-values under TCDD-treated groups are from Fisher's exact test.

Table 6-4. Tumor Incidences in Male and Female B6C3F1 Mice Given TCDD by Gavage for 2 Years[a,b]

Target organ/tumor type	Sex	Dose (ng/kg/day)			
		0	1.4	7.1	71
Liver carcinoma	male	8/73 p=0.002	9/49 p=0.19	8/49 p=0.28	17/50 p=0.002
adenoma		7/73 p=0.024	3/49 --	5/49 p=0.6	10/50 p=0.09
Lung adenoma or carcinoma		10/71 p=0.004	2/48 --	4/48 --	13/50 p=0.08
Subcutaneous fibrosarcoma	female	1/74 p=0.007	1/50 p=0.6	1/48 p=0.6	5/47 p=0.032
Liver carcinoma		1/73 p=0.008	2/50 p=0.4	2/48 p=0.4	6/47 p=0.014
adenoma		2/73 p=0.11	4/50 p=0.2	4/48 p=0.2	5/47 p=0.08
Thyroid follicular cell adenoma		0/69 p=0.016	3/50 p=0.07	1/47 p=0.4	5/46 p=0.009
Lymphoma		18/74 p=0.011	11/50 --	13/48 p=0.4	20/47 p=0.029

[a]Source: NTP, 1982.
[b]p-Values for controls represent Cochran-Armitage trend test, and p-values for TCDD-treated groups are derived from Fisher's exact test.

the lowest-observed-effect level (LOEL) is 1.4 ng/kg/day and a NOEL was not achieved within the specified dose range, suggesting that thyroid tumor incidence may be the most sensitive site for TCDD-mediated carcinogenesis. Because 70 ng/kg/day is above the maximum-tolerated dose (MTD) (Huff et al., 1991), thyroid tumors occur at doses more than 50 times lower than the MTD.

TCDD-induced neoplasms of the adrenal gland were observed in the 7.1 ng/kg/day/dose group in male rats and in high-dose female rats. Fibrosarcomas of the subcutaneous tissue were significantly elevated in high-dose female mice and female rats. One additional tumor type, lymphoma, was seen in high-dose female mice. Lung tumors were elevated in high-dose female mice; the increase was not statistically significant when compared with concurrent controls, but the increase was dose related (Cochran-Armitage trend test, $p=0.004$).

Therefore, TCDD is a multisite complete carcinogen (Huff, 1992) and induced neoplasms in rats and mice of both sexes. As in Kociba et al. (1978), liver tumors were observed with greater frequency in treated female rats, but in male rats, the thyroid appears to be the most sensitive (increased tumor incidence at doses as low as 1.4 ng/kg/day).

6.2.3. Syrian Golden Hamster

Groups of 10 to 24 male Syrian Golden hamsters were given two to six intraperitoneal or subcutaneous injections of TCDD over a 4-week period at doses of 0, 50, or 100 μg/kg TCDD in dioxane (Rao et al., 1988). The experiments were terminated after 12 to 13 months. The 100 μg/kg groups (total dose of 600 μg/kg) from both injection routes developed squamous cell carcinomas of the skin in the facial region: 4/18 (22%) from the intraperitoneal injection and 3/14 (21%) from the subcutaneous injection. The lesions were large (1.5 to 3 cm) with extensive necrosis, and some metastasized to the lung. The earliest neoplasms were detectable 8 months after the initial injection. Similar lesions were not seen in hamsters receiving two intraperitoneal injections of 100 μg/kg TCDD or six subcutaneous injections of dioxane vehicle, and none have been reported over the past 10 years in this laboratory. An extensive study by Pour et al. (1976) identified only one skin papilloma in 533 control Syrian hamsters. This report demonstrates that the hamster, a nonresponsive

species (for acute toxic effects), is susceptible to the carcinogenic actions of TCDD at doses well below the maximum-tolerated dose.

6.2.4. B6C3 and B6C Mice

In a study by Della Porta et al. (1987), TCDD was administered intraperitoneally in corn oil at doses of 0, 1, 30, and 60 μg/kg to groups of 89 to 186 B6C3 and B6C mice of both sexes once weekly for 5 weeks starting at day 10 of life, and the animals were observed until 78 weeks of age. Histopathological observations were limited to the liver, kidney, and organs with apparent or suspected pathological changes. Thymic lymphomas were induced at the 60 μg/kg level in both sexes of both hybrids and at 30 μg/kg in all but female B6C3 mice. Neoplasms of the liver occurred in male B6C3 mice at 30 μg/kg and female B6C3 mice at 60 μg/kg. In a separate experiment, groups of 42 to 50 B6C3 mice were exposed to 0, 2.5, and 5.0 μg/kg TCDD in corn oil by gavage once weekly for 52 weeks starting at 6 weeks of age. The study was stopped at 110 weeks. Increased incidences of liver tumors were related to TCDD exposure at both dose levels.

In summary, there is convincing evidence in the scientific literature that TCDD is a potent multisite carcinogen in both sexes of several species, and carcinogenic effects have been observed at doses that are orders of magnitude less than the maximum-tolerated dose.

6.2.5. Carcinogenicity of Related Compounds

A mixture of two isomers of hexachlorodibenzo-*p*-dioxin (HCDD) (1,2,3,6,7,8 and 1,2,3,7,8,9) was given by gavage twice weekly for 2 years to Osborne-Mendel rats and B6C3F1 mice (NTP, 1980). The doses of HCDD were 0, 1.25, 2.5, or 5 μg/kg/week in rats and male mice. Doses for female mice were 0, 2.5, 5, and 10 μg/kg/week. There was no effect of administration of HCDD on survival of either sex of rats or mice (NTP, 1980). Results revealed that HCDD increased liver tumors in both sexes of rats and mice although female rats seemed to be more sensitive than male rats (significant increases detected in female rats in the 1.25 μg/kg/week dose group, equivalent to 180 ng/kg/day). Therefore, HCDD is approximately 1/20 as potent a liver carcinogen as TCDD.

Dermal applications of the same HCDD mixture as described above (NTP, 1982b) were given to Swiss Webster mice for 104 weeks (three weekly). For the first 16 weeks, doses of 5 ng/application were used. Thereafter, doses of 10 ng/application were used. No HCDD-exposure-related carcinogenic responses were noted.

Dibenzo-*p*-dioxin given in the diet for 2 years at concentrations of 0, 5,000, and 10,000 ppm did not increase carcinogenic responses in Osborne-Mendel rats or B6C3F1 mice (NCI, 1979a). 2,7-Dichlorodibenzo-*p*-dioxin (DCDD) in the diet of Osborne-Mendel rats for 110 weeks or B6C3F1 mice for 90 weeks at levels of 0, 5,000, or 10,000 ppm did not increase neoplasms in male or female rats or in female mice. In male mice, increased incidences of lymphoma or hemangiosarcoma were observed in the low-dose group and neoplasms of the liver were observed in both dose groups (NCI, 1979b). The more highly chlorinated dibenzo-*p*-dioxins (CDDs) and dibenzofurans (CDFs) have not been studied in long-term animal cancer bioassays. Many of the CDDs and CDFs bioaccumulate and exhibit toxicities similar to those of TCDD and are considered to be carcinogens (EPA Science Advisory Board, 1989).

6.3. MECHANISMS OF TCDD CARCINOGENICITY

There is substantial evidence that TCDD is not a direct genotoxic agent. Because "genotoxic" and "nongenotoxic" are controversial and often misused terms, it is prudent to describe accurately the scientific criteria used to call a chemical "genotoxic" or "nongenotoxic" (IARC, 1992). Some of the criteria for designating TCDD a nongenotoxic agent are that it does not bind covalently to DNA (does not form DNA adducts). Although one study detected radioactivity associated with crude DNA preparations after in vivo exposure, no study that has rigorously looked for TCDD-DNA adducts has been positive. TCDD is negative in short-term tests for genotoxicity and is a potent promoter and weak initiator in multistage models for chemical carcinogenesis. In a recent study (Turteltaub et al., 1990) using accelerator mass spectrometry, DNA adducts were not detected in rodent tissue following exposure to TCDD. This method is extraordinarily sensitive, being capable of detecting one adduct in 10^{12} normal nucleotides. Randerath et al. (1988) were unable to detect TCDD-related DNA adducts by the sensitive ^{32}P postlabeling method (limit of

detection of one adduct in 10^9 normal nucleotides). For comparison, approximately one adduct in 10^6 normal nucleotides is found in rodent tissues following carcinogenic doses of benzo(a)pyrene (7,8-diol-9,10 epoxide deoxyguanosine DNA adduct), methylnitrosourea (0^6 methylguanine), or NNK (0^6 methylguanine).

Another criterion for designating TCDD a "nongenotoxic carcinogen" is that numerous studies have demonstrated that TCDD is negative in the *Salmonella*/Ames test in the presence or absence of a mixed-function oxidase (MFO) activating system. These negative studies have encompassed 13 different bacterial strains with tests performed in 9 laboratories (Wassom et al., 1977; Kociba, 1984; IARC, 1982; Giri, 1987; Shu et al., 1987). Using its battery of tests for genetic toxicity, the NTP (1984) concluded that TCDD was nonmutagenic. Additionally, several scientific panels have stated that false negatives for TCDD genetic toxicity are highly unlikely (EPA Science Advisory Board, 1984). TCDD has been found to promote the transformation of C3H/10T1/2 cells; it was concluded that this response did not reflect TCDD's ability to directly damage DNA (Abernethy et al., 1985). In human populations accidentally or occupationally exposed to TCDD, there is no consistent evidence for increased frequencies of chromosomal aberrations in workers exposed to TCDD (Shu et al., 1987).

Although TCDD is negative in genetic toxicity tests, recent reports have demonstrated that high doses of TCDD (50 to 100 μg/kg) induce single-strand breaks in Sprague-Dawley rats, presumably as a consequence of increased lipid peroxidation (Wahba et al., 1988, 1989). In another set of studies, increased frequency of sister chromatid exchanges was observed in lymphocytes of people exposed to pentachlorinated dibenzo-*p*-dioxins (PCDFs) in Taiwan when those lymphocytes were challenged with α-naphthoflavone (Lundgren et al., 1986, 1988). The possible mechanism responsible for this effect is that the PCDFs cause increased rates of metabolic activation of α-naphthoflavone to DNA reactive metabolites (Lundgren et al., 1987). These findings are consistent with the idea that TCDD's ability to induce drug-metabolizing enzymes (CYP1A1 and 1A2) may lead to an increased rate of formation of DNA reactive metabolites of some carcinogens, most notably the polycyclic aromatic hydrocarbons (PAHs) and aromatic amines. However, there is evidence that the opposite effect occurs in some cases because in vivo exposure to CYP1A1 inducers actually

leads to a decrease in DNA adducts in target tissue following in vivo exposure to PAHs such as benzo(a)pyrene (Cohen et al., 1979; Parkinson and Hurwitz, 1991). It can reasonably be concluded that TCDD exposure may increase the rate of DNA adduct formation for some carcinogens but decreases the rate for others and that predictions should not be made without experimental data on DNA adduct concentrations in control and TCDD-treated animals.

A final criterion for designating TCDD a nongenotoxic carcinogen is that it is a potent tumor promoter and a weak initiator or noninitiator in two-stage models for liver (Pitot et al., 1980; Graham et al., 1988; Lucier et al., 1991; Clark et al., 1991a; Flodstrom and Ahlborg, 1991) and skin (Poland et al., 1982). These findings are discussed in more detail in the section on tumor promotion, including plausible mechanisms for the tumor-promoting actions of TCDD such as TCDD-mediated increases in cell proliferation rates of genetically altered cells. However, a recent report by Yang et al. (1992) has demonstrated that immortalized human keratinocytes cultured with TCDD were neoplastically transformed, as evidenced by tumorigenic activity of those cells in nude mice. This response is characteristic of genotoxic carcinogens and occurred at a low TCDD concentration (0.1 nm). For comparison, induction of CYP1A2 in these same cells was not detected until a dose of 3 nm was used (Yang et al., 1992).

It is now accepted by the scientific community that most if not all of TCDD's toxic and biochemical effects, including tumor promotion, are Ah receptor dependent and that TCDD provides an example for evaluating the issues relevant to risk assessment for receptor-mediated carcinogens. The steps involved in Ah receptor-mediated events are reviewed in Chapter 2, Mechanisms of Toxic Actions.

6.4. INITIATION/PROMOTION STUDIES

The multistage nature of chemical carcinogenesis is being defined by an increasing understanding of the discrete steps required to produce a genetically altered cell that is clonally expanded and ultimately progresses to a tumor (IARC, 1992; Barrett and Wiseman, 1987; Swenberg et al., 1987; Barrett, 1992) (Figure 6-1). Briefly, the process involves damage to a specific site on DNA, a round of cell replication to fix that damage into the genome, clonal expansion of the genetically altered cells (tumor promotion), followed by

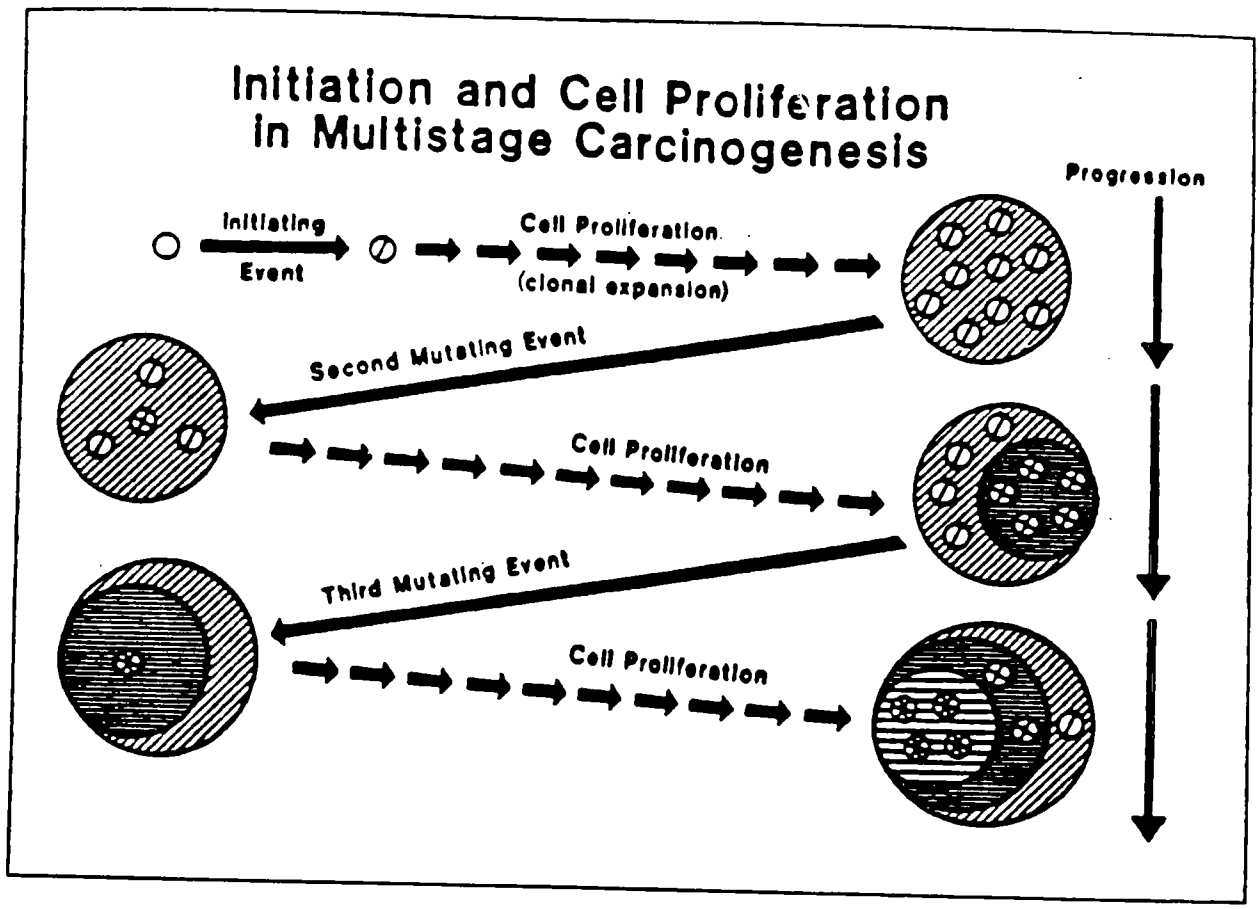

Figure 6-1. Schematic representation of multistep carcinogenesis including the roles of genetic damage and cell proliferation. It is important to note that several DNA-damaging steps and several cell proliferation steps are likely to be involved during the complete process of chemical carcinogenesis.

Source: Swenberg et al., 1987.

additional genetic damage and rounds of cell replication (tumor progression). Figure 6-1 schematizes the multistage nature of cancer. The birth and death rates of genetically altered cells compared with normal cells are the centerpiece of risk assessment models that recognize the multistage nature of chemical carcinogenesis (Moolgavkar and Knudson, 1981; Portier, 1987). The roles of protooncogene activation and tumor suppression gene inactivation have provided clues in attempts to discern discrete steps in carcinogenesis. It is also clear that cell proliferation is an essential component of chemical carcinogenesis, for without it DNA damage would not be fixed into the genome and clonal expansion of genetically altered cells would not occur.

Concurrent with our increased understanding of the mechanistic underpinnings of chemical carcinogenesis, multistage models have been developed to identify the particular stage or stages in which carcinogens act to increase tumor incidence. There is a wealth of information on liver initiation/promotion protocols in the scientific literature (Pitot and Sirica, 1980; Farber, 1984; Pitot and Campbell, 1987). These protocols frequently employ a single initiating dose of a chemical that damages DNA, followed by enhancement of cell replication (partial hepatectomy or cytotoxicity) to fix that damage into the genome (initiation), and then chronic exposure to a chemical that produces clonal expansion of the genetically altered cells (promotion). Increased tumor incidence is produced by chemicals that act at either stage. It is important to note that "initiation" and "promotion" are operational and not mechanistic terms because both stages are likely to be composed of multiple steps, and the mechanisms are not mutually exclusive. Nevertheless, the protocols have provided valuable information in our attempts to understand chemical carcinogenesis. Detailed descriptions of initiation/promotion protocols in liver and skin are provided elsewhere (Pitot and Campbell, 1987; Dragan et al., 1991; Pitot et al., 1987; Farber, 1984; Slaga et al., 1982; Peraino et al., 1981; Ito et al., 1980).

6.4.1. Two-Stage Models in Rat Liver

Pitot et al. (1980) reported that TCDD was a potent liver tumor promoter when rats were initiated with a single dose of DEN followed by chronic TCDD exposure (0.14 and 1.4 μg/kg subcutaneously once every 2 weeks for 7 months). These doses are equivalent to 10

and 100 ng TCDD/kg/day (the medium and high dose in the Kociba bioassay). Histological evaluation revealed that five of seven animals that had received DEN and the high TCDD dose had hepatocellular carcinomas. No liver tumors were evident in rats receiving DEN only, DEN/low-dose TCDD, or TCDD only (high or low dose). Enzyme-altered foci (EOF) in liver also were evaluated in this study, and these are considered to represent preneoplastic lesions because increases in EOF are associated with liver cancer in rodents (Maronpot et al., 1989; Popp and Goldsworthy, 1989; Pitot et al., 1989; Williams, 1989). The EOF data were consistent with the tumor data in that a large proportion of the liver was occupied by preneoplastic lesions (43%) in animals receiving DEN and the high dose of TCDD. A much smaller proportion of the liver was occupied by EOF in the other groups. This work provides strong evidence that TCDD is a potent tumor promoter in liver.

A second set of studies (Graham et al., 1988; Lucier et al., 1991; Clark et al., 1991a; Dragan et al., 1992) have confirmed and extended Pitot's findings, including data on the mechanistic basis for TCDD's tumor-promoting effects in rat liver. These studies also used DEN as the initiator and have demonstrated that TCDD's liver tumor-promoting actions are ovarian dependent. This finding is consistent with 2-year bioassays showing that TCDD is a hepatocarcinogen in female rats but not in male rats. In the tumor-promoting studies (Graham et al., 1988; Lucier et al., 1991), DEN was used as the initiating agent and TCDD (biweekly doses of 1.4 μg TCDD/kg, equivalent to 100 ng/kg/day for 30 weeks) was used as the promoter. There were four groups of intact female rats (controls, TCDD only, DEN only, and DEN/TCDD). The same four groups were used following ovariectomy. Data revealed that TCDD was a much weaker liver tumor promoter in ovariectomized rats (Table 6-5). For example, there were 387 gamma glutamyl transpeptidase (GGT) foci/cm^3 in intact rats compared with 80 in ovariectomized rats in the DEN/TCDD groups. Corresponding differences were evident in the proportion of liver occupied by GGT foci; 0.37% in DEN/TCDD intact rats compared with 0.08% in DEN/TCDD ovariectomized rats. Few or no foci were found in the control or TCDD-only groups. Placental glutathione transferase (PGT) is being used increasingly as a phenotypic marker of enzyme-altered foci (Ito et al., 1989), and results with this marker of preneoplasia were similar to those for GGT in that ovariectomy protected against the liver tumor-promoting actions of TCDD.

Table 6-5. Preneoplastic Foci and Cell Proliferation After 30 Weeks of TCDD as Tumor Promoter[a]

	S/C[b]	S/TCDD	DEN/C	DEN/TCDD
GGT + foci/cm^3				
intact	6	5	44	387[c]
ovariectomized	0	0	30	80
GGT + foci (vol. fraction)[c]				
intact	0.01	0.01	0.03	0.37[c]
ovariectomized	0	0	0.03	0.08
BrdU-labeling index[d]				
intact	0.3[c]	6.0[c]	0.8	7.3[c]
ovariectomized	1.1	1.0	1.1	0.7

[a]Source: Clark et al., 1991.
[b]S/C = Controls; S/TCDD = TCDD only; DEN/C = DEN only, no TCDD; DEN/TCDD = DEN initiated and TCDD promoted.
[c]Significantly different from ovariectomized.
[d]Percentage of hepatocytes undergoing replicative DNA synthesis in 1 week following 30 weeks of TCDD exposure.

The influence of ovariectomy on liver tumor incidence was evaluated in a parallel experiment using the same treatment groups in which TCDD was administered for 60 weeks. In the intact DEN/TCDD rats, liver tumor incidence was 13/37, with a total of 32 tumors compared with 7/39 (11 total tumors) in DEN/TCDD ovariectomized rats. Both hepatocellular adenomas and carcinomas were evident, along with a smaller incidence of hepatocholangiomas and hepatocholangiocarcinomas.

The mechanisms responsible for the protective effect of ovariectomy are not clear, but ovarian influences on liver TCDD retention do not seem to be involved; liver TCDD concentrations were ~20 ppb in both intact and ovariectomized rats (Lucier et al., 1991), which is similar to liver concentrations reported by Kociba et al. (1978) using the same dose of TCDD (100 ng/kg/day) but for 2 years rather than 60 weeks. One plausible mechanism may be related to cell proliferation: TCDD did not stimulate cell proliferation rates in ovariectomized rats whereas a mean increase of tenfold was apparent in intact rats receiving 100 ng TCDD/kg for 30 weeks (Table 6-5) (Lucier et al., 1991). There was considerable interindividual variation in both cell proliferation rates and enzyme-altered foci in the DEN/TCDD groups. Comparisons of the two data sets revealed a strong positive correlation between enzyme-altered foci and cell proliferation, although the importance of this finding is diminished by the fact that cell proliferation was quantified in nonlesioned hepatocytes. The mechanism whereby ovarian hormones and TCDD interact to produce cell proliferation in hepatocytes may involve growth factor pathways. Consistent with this idea, TCDD produced a loss of plasma membrane epidermal growth factor (EGF) receptor in intact rats but not ovariectomized rats (Clark et al., 1991a). EGF is thought to provide a mitogenic stimulus in hepatocytes and play a key role in hepatocarcinogenesis (Vickers and Lucier, 1991; Velu, 1990; Shi and Yager, 1989; Eckl et al., 1988). A schematic representation of a plausible mechanism for the role of estrogen in TCDD-mediated liver cancer in rats is given in Figure 6-2.

Another possible mechanism for the influence of the ovaries is that TCDD induces cytochrome P-4501A2, which could lead to DNA reactive metabolites of 17β-estradiol, the naturally occurring estrogen. P-4501A2 catalyzes the formation of catechol estrogens that are considered by some to be DNA reactive precursors (Metzler, 1984; Li and Li, 1990).

POSSIBLE SEQUENCE OF EVENTS INVOLVED IN ESTROGEN-DEPENDENT TCDD PROMOTION OF LIVER TUMORS

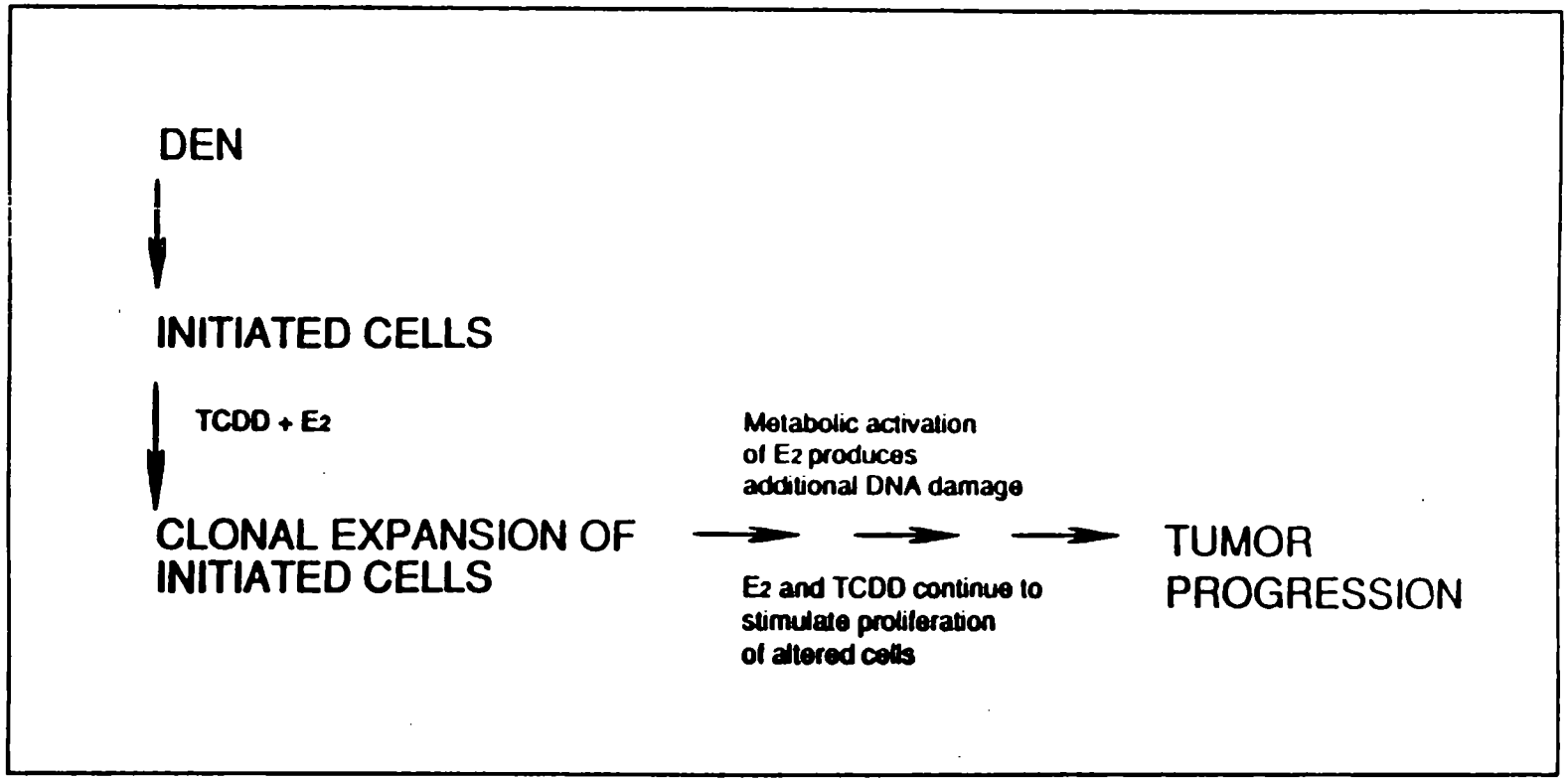

Figure 6-2. Operational model of TCDD/estrogen interactions relative to tumor promotion in a two-stage model of hepatocarcinogenesis. Clonal expansion of initiated cells may reflect stimulation of mitogenesis through receptor-mediated events involving epidermal growth factor receptor, estrogen receptor, and the Ah receptor.

Source: Vickers and Lucier, 1991.

The chlorinated dibenzofurans and other chlorinated dibenzo-*p*-dioxins are also liver tumor promoters. In a recent study (Flodstrom and Ahlborg, 1992), enzyme-altered foci were increased in female Sprague-Dawley rat livers by an initiating dose of DEN followed by TCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, or 2,3,4,7,8-pentachlorodibenzofuran used as the promoting agent. Comparative potencies indicated that the two CDDs were nearly equipotent and the PCDF was about 1/10th as potent as TCDD. These results are consistent with the idea that the hepatocarcinogenic actions of TCDD and its structural analogs are Ah receptor dependent.

6.4.2. Rat Lung

Because the lung and respiratory tract seem to be target sites for TCDD carcinogenesis in humans (Fingerhut et al., 1991), it is of interest to evaluate whether TCDD is a tumor promoter in rodent lung. The only published report on lung tumors used DEN as the initiating agent and TCDD (100 ng/kg/day for 60 weeks) as the promoting agent (Clark et al., 1991a). Both intact and ovariectomized rats were used, and the results were surprising. In contrast to liver tumor promotion, lung tumors were seen only in DEN/TCDD ovariectomized rats (4/37). No lung tumors were present in DEN/TCDD intact rats, in DEN only/TCDD only, or in control rats with or without ovariectomy. The background incidence of lung tumors in rats is very low so the lack of tumors in controls was not unexpected (Haseman et al., 1984). The four tumors in DEN/TCDD intact rats were composed of two squamous cell carcinomas and two adenocarcinomas.

The rodent tumorigenicity data provide clues to the complex hormonal interactions that produce site-specific carcinogenic actions of TCDD. Liver tumors are ovarian dependent, whereas the ovaries appear to protect against TCDD-mediated tumor promotion in lung. Therefore, the rat tumor data are of interest because recent epidemiologic studies (Chapter 7) have shown that TCDD exposure is associated with an increase in respiratory tract tumors.

6.4.3. Mouse Skin

Initiation/promotion studies on skin have demonstrated that TCDD is a potent tumor promoter in mouse skin as well as rat liver. Poland et al. (1982) administered a single

dermal initiating dose of *N*-methyl-*N*-nitrosoguanidine (MNNG) to hairless mice followed by twice weekly doses of TCDD (3.75, 7.5, 15, or 30 ng) or TPA (1 or 3 μg) for 20 weeks. TCDD promoted the development of papillomas at all doses, and the response was dose dependent (100% of the animals had tumors in the high-dose TCDD group). Control animals or animals receiving MNNG or TCDD only exhibited only a low incidence of tumors. These studies demonstrate that TCDD is at least two orders of magnitude more potent an agent than TPA in mouse skin (Poland et al., 1982). Based on structure activity and genetic studies, it appears that the skin tumor-promoting actions of TCDD are Ah receptor dependent. Moreover, tumorigenic responses segregate with the hr locus, and biochemical responses such as CYP1A1 induction can occur without carcinogenesis (Poland and Knutson, 1982; Poland et al., 1982).

Other studies have tested TCDD as an initiator and TPA as a promoter in CD-1 mice (DiGiovanni et al., 1977). Results revealed that TCDD had weak or no initiating activity in this system. To better understand the possible influence of TCDD-mediated induction of cytochrome P-450 on the carcinogenicity of PAHs, TCDD was coadministered with benzo(a)pyrene or dimethylbenzanthracene to mice followed by promotion with TPA (Cohen et al., 1979). Results revealed that TCDD decreased tumor incidence of both PAHs compared with controls. However, coadministration of TCDD with 3-methylcholanthrene to mice produced tumor incidences similar to those produced by 3-methylcholanthrene alone (Kouri et al., 1978). These results are consistent with the findings that TCDD induction of drug-metabolizing enzymes is associated with both metabolic activation as well as deactivation of PAHs (Lucier et al., 1979).

The relative toxicity and tumor-promoting capacity of two CDFs (2,3,4,7,8-CDF and 1,2,3,4,7,8-CDF) have been investigated in hairless mice (Hebert et al., 1990). These studies used a treatment protocol similar to that of Poland et al. (1982), including the use of MNNG as the initiating agent and varying doses of TCDD, 2,3,4,7,8-CDF, or 1,2,3,4,7,8-CDF for 20 weeks. Proliferative lesions (squamous cell papilloma, squamous cell carcinoma, or hyperproliferative nodules) were quantified. Results demonstrated that 2,3,4,7,8-CDF was 0.2 to 0.4 times as potent as TCDD and that 1,2,3,4,7,8-CDF was 0.08 to 0.16 times as potent as TCDD. These data suggest that the tumor-promoting potencies of

structural analogs of TCDD, like the promotion of liver tumors, reflect relative binding properties to the Ah receptor. However, this is an effect of chronic exposure; therefore, rates of metabolism/clearance would obviously affect correlations between Ah receptor binding and tumor promotion.

Taken together, results on initiation/promotion protocols indicate that TCDD is an extraordinarily potent promoter of liver and skin tumors (Pitot et al., 1987), and the results provide strong evidence that the carcinogenic actions are Ah receptor mediated. A summary of studies on tumor promotion by TCDD or the polychlorinated dibenzofurans is given in Table 6-6. Plausible mechanisms of actions responsible for the tumor-promoting actions of TCDD and the impact of these mechanisms on dose-response relationships are presented in the next section.

6.5. BIOCHEMICAL RESPONSES

The list of biochemical effects produced by TCDD in humans, experimental animals, and cell systems is expanding. These effects include those that may alter normal cell regulatory processes such as cell proliferation and differentiation, metabolic capacity, and hormonal pathways. This section on biochemical responses will summarize some of the changes produced by TCDD, including discussion of (a) possible relevance of the response to TCDD-mediated cancer, (b) whether the response is Ah receptor mediated, (c) whether information is available on the role of transcriptional activation, (d) dose-response relationships, and (e) whether animal models are consistent with human responses. This chapter will not attempt to evaluate all of the biochemical and molecular responses to TCDD but will focus on the ones that are either the most relevant to carcinogenic responses or have received the most study. The responses selected for evaluation are cytochrome P-4501A1 (CYP1A1), cytochrome P-4501A2 (CYP1A2), EGFR, estrogen receptor (ER), and UDP-glucuronosyltransferase (UDPGT). Table 6-7 lists many of the biochemical changes produced by TCDD in in vivo and/or in vitro systems and some information on mechanisms of action.

Table 6-6. Summary of Positive Tumor-Promoting Studies on TCDD and CDFs

Species/Sex	Initiator	Promoter	Site	Reference
Rat/female	DEN	TCDD	Liver	Pitot et al., 1980
Rat/female	DEN	TCDD	Liver	Graham et al., 1988
Rat/female	DEN	TCDD	Liver	Lucier et al., 1991
Rat/female	DEN	TCDD	Liver	Clark et al., 1991a
Rat/female	DEN	TCDD	Liver	Flodstrom and Ahlborg, 1991
Rat/female	DEN	TCDD	Liver	Flodstrom and Ahlborg, 1992
Rat/female	DEN	PCDFs	Liver	Flodstrom and Ahlborg, 1992
Rat/female	DEN	TCDD	Liver	Dragan et al., 1992
Rat/female	DEN	TCDD	Liver	Lucier et al., 1992
Mice/female hairless	MNNG	TCDD	Skin	Poland et al., 1982
Mice/female hairless	MNNG	PCDFs	Skin	Hebert et al., 1990
Rat/female (ovariectomized)	DEN	TCDD	Lung	Clark et al., 1991a

Table 6-7. Classification of Members of the Ah Gene Battery[a]

Class	Gene/Product	Secreted protein
Activation of gene transcription; Ah receptor-mediated	CYP1A1, Cytochrome P1450	-
	Gst-Ya, glutathione S-transferase	-
	Nmo-1, menadione oxidoreductase	-
Activation of gene transcription; AhR agonist-mediated	Clone 1, unknown gene	?
	CYP1A2, cytochrome P2450	-
	PAI-2, plasminogen activator inhibitor-2	+
	T-ALDH, aldehyde deydrogenase	-
Induction of mRNA levels; AhR agonist-mediated	Clone 141, unknown gene	?
	c-erb A related, hormone receptor	-
	GST-Yb, glutathione S-transferase	-
	GST-Yc	-
	αhCG, human chorionic gonadotropin	+
	IL-β, interleukin-1β	+
	MDR-1, multidrug resistance	-
	Testosterone 7 α-hydroxylase	-
	TGF-α, transforming growth factor-α	+
Induction of enzyme activity; Ah receptor-mediated	ODC, ornithine decarboxylase	-
	Ugt-1, UDP-glucuronyl transferase	-
	EGFR, epidermal growth factor receptor	-
	ER, estrogen receptor	-
	Gastrin	+
	TNF-α, tumor necrosis factor-α	+
Induction of enzyme activity; AhR agonist-mediated	ALAS, δ-aminolevulinic acid synthetase	-
	Aryl hydrocarbon-binding protein	-
	Choline kinase	-
	60-kd microsomal esterase	-
	Malic enzyme	-
	Phospholipase A_2	-
	Protein kinase C	-
	Enzyme $pp60^{c\text{-}src}$, tyrosine kinase	-

[a]Source: Sutter and Greenlee, 1992.

6.5.1. CYP1A1 and 1A2

The most studied response to TCDD has been induction of cytochrome P-450 isozymes (Whitlock, 1990; Silbergeld and Gasiewicz, 1989; Poland and Knutson, 1982). The first reports of P-450 induction in vivo and in vitro appeared in 1973 (Lucier et al., 1973; Greig and DeMatteis, 1973; Poland and Glover, 1973), and hundreds of papers have been published on the subject since that time. These papers have dealt with various aspects of TCDD-mediated induction of P-450, such as isozyme specificity, time course, structure-activity relationships, molecular mechanisms of transcriptional activation of the CYP1A1 gene, identification of transcriptional activating factors, tissue and cell specificity, and dose-response relationships. The molecular mechanisms responsible for enzyme induction are described in the chapter by Whitlock in this volume.

The mechanistic relationship of CYP1A1 and 1A2 induction to cancer or any other toxic end point following dioxin exposure has not been demonstrated, yet considerable controversy exists on this subject (Roberts, 1991). Since CYP1A1 functions to catalyze the metabolic activation of many chemicals such as the polycyclic aromatic hydrocarbons to DNA reactive metabolites, it has been postulated that induction of CYP1A1 might enhance the carcinogenic actions from a given exposure level to many PAHs. Usually, however, preinduction of CYP1A1 diminishes the carcinogenic potency of PAHs such as 3-methylcholanthrene, benzo(a)pyrene, and 7,2-dimethylbenzanthracene if exposure to the inducing agent is short term (Parkinson and Hurwitz, 1991; Wattenberg, 1985; Cohen et al., 1979; Wattenberg, 1978; Miller et al., 1958). Induction also protects against the carcinogenic actions of aflatoxin, diethylnitrosamine, arylamines, and urethane. Protection occurs at numerous cancer sites including liver and lung. Several lines of evidence support the idea that enzyme induction is the mechanism responsible for the protective effect. First, treatment of mice, deficient in the Ah receptor, with inducers does not protect against PAH-mediated cancer (Kouri et al., 1978). Second, the ability of inducing agents to protect against cancer is positively correlated with their potency as inducing agents (Wattenberg and Leong, 1970; Arcos et al., 1961). Third, the inducing agent must be administered at least 1 day prior to treatment, which allows sufficient time for the inducer to produce elevated levels of CYP1A1 (Parkinson et al., 1983; Wheatley, 1968).

The most probable mechanism responsible for the protective effect of enzyme induction is that it leads to decreased concentrations of promutagenic DNA adducts in target tissues. These findings appear to contradict the knowledge that CYP1A1 is required for the metabolism of PAHs, aflatoxin, and several other carcinogens to DNA reactive arene oxides (Guengerich, 1988; Levin et al., 1982; Conney, 1982). For example, the promutagenic DNA adduct of benzo(a)pyrene appears to be a 7,8-diol-9,10 epoxide metabolite adducted to deoxyguanosine, and formation of this metabolite requires two separate actions of CYP1A1. The contradiction can be resolved by analysis of the entire metabolic pathways for chemical carcinogens whose potencies are decreased by pretreatment with inducing agents. In addition to CYP1A1-mediated increases in metabolic activation, this cytochrome also converts PAHs to inactive metabolites (Thakker et al., 1985; Pelkonnen and Nebert, 1982). Moreover, induction of uridine diphosphoglucuronyltransferase also occurs coordinately with CYP1A1 induction (Lucier et al., 1986). This enzyme also detoxifies metabolites of PAHs and other carcinogens and facilitates their excretion from the body (Thakker et al., 1985; Nemoto and Gelboin, 1976). Therefore, it appears that TCDD-mediated enzyme induction increases the rate of detoxification of some carcinogens to a greater extent than it increases the rate of formation of DNA-damaging metabolites.

Although there is no clear mechanistic link between CYP1A1 induction and cancer, it is important to note that many CYP1A1 inducers are themselves carcinogens when encountered in chronic dosing regimens; therefore, the protective effect of inducing agents appears to be limited to short-term exposure. For example, benzo(a)pyrene, 3-methylcholanthrene, and TCDD are CYP1A1 inducers and multisite carcinogens (Vanden Heuvel and Lucier, 1993; Levin et al., 1982; Slaga et al., 1979; Sims and Glover, 1974).

The relationship of CYP1A2 induction to the carcinogenic actions of other compounds is less clear than it is for CYP1A1. For example, CYP1A2 catalyzes the formation of catechol estrogens from 17β-estradiol (Graham et al., 1988). The catechol estrogens are considered to be possible toxic metabolites in that they could lead to increased free radical damage to cellular macromolecules such as DNA (Li and Li, 1990; Metzler, 1984). This mechanism could be responsible, in part, for the findings that TCDD is a hepatocarcinogen in female rats but not male rats and that ovariectomy protects against the hepatocarcinogenic

actions of TCDD. Also consistent with the hepatocarcinogenicity data is the observation that CYP1A2 is induced in liver but not in extrahepatic organs, with the possible exception of the nasal mucosa (Goldstein and Linko, 1984). In contrast, CYP1A1 induction occurs in virtually every tissue of the body, which is consistent with the observation that the Ah receptor is found in a wide variety of cell types.

There are a number of studies described in the scientific literature on dose-response relationships for TCDD's effects on CYP1A1 and 1A2 (DeVito et al., 1991; Lin et al., 1991a; Kedderis et al., 1991; Harris et al., 1990b; Goldstein and Safe, 1989; Abraham et al., 1988; Lucier et al., 1986, 1973; Vecchi et al., 1983; Kitchin and Woods, 1979; Poland and Glover, 1973). These studies include single and chronic dosing schedules (Tritscher et al., 1992; Graham et al., 1988; Sloop and Lucier, 1987), time-course evaluations, and species comparisons. Dose-response relationships have been evaluated by quantitation of CYP1A1- and 1A2-dependent enzyme activities, quantitation of mRNA levels by Northern blot analysis, and quantitation of CYP1A1 and 1A2 protein by radioimmunoassay and also by immunolocalization in tissue sections. All of the above methods have yielded consistent results. The single-dose ED_{50} for CYP1A1 or 1A2 induction is approximately 0.5 to 1.5 μg TCDD/kg in both rats and mice. In a chronic exposure situation, the ED_{50} is in the range of 5 to 10 ng/kg/day (Tritscher et al., 1992). The limit of detection for enzyme induction varies depending on the method used for quantitation; that is, P-450-dependent enzyme activities, mRNA, or protein. Recently, it was shown (Kohn et al., 1993) that TCDD-mediated increases in CYP1 in mRNA were detectable following a single dose of 0.1 ng/kg, which produces a TCDD liver concentration equivalent to a chronic dose of 2 to 5 pg/kg/day.

Evaluations of various data sets for TCDD-mediated dose-response relationships have revealed some interesting information. One way of analyzing data for linearity or nonlinearity of dose response for receptor-mediated events is the Hill equation (Hayashi and Sakamoto, 1986). A Hill coefficient of 1 suggests a linear relationship between exposure and dose throughout the experimental dose range and would predict a proportional relationship between target tissue concentration of TCDD and biological response at all dose levels. This would imply that the response had no practical threshold or "no effect level." Hill

coefficients greater than 1 would indicate sublinearity in dose response, whereas a Hill coefficient of less than 1 would indicate supralinearity for response in the low-dose region. Analyses of both single exposure as well as chronic exposure data for CYP1A1 and CYP1A2 induction in rat or mouse liver indicate a Hill coefficient of slightly greater than 1 for CYP1A1 and slightly less than 1 for CYP1A2 (Portier et al., 1993; Kohn et al., 1993). Although these analyses involve an extrapolation beyond the range of experimental data, they are consistent with the hypothesis that there is no threshold for TCDD-mediated induction of CYP1A1 and 1A2.

Immunological detection of induced CYP1A1 and 1A2 in liver sections obtained from rats exposed chronically to TCDD suggests hepatocyte heterogeneity in response to TCDD (Tritscher et al., 1992; Bars and Elcombe, 1991). For example, relatively low doses of TCDD (1 ng/kg/day) appear to maximally induce some cells around the centrilobular region. Increasing doses of TCDD increase the number of cells responding rather than the amount of induction in responding cells. These data, which document cell differences in sensitivity to induction, complicate evaluation of dose-response relationships. For example, some hepatocytes appear to be maximally induced by low doses of TCDD, whereas other hepatocytes exhibit no detectable P-450 induction response at these same doses. As discussed earlier, a mechanistic link between P-450 induction and cancer has not been established. Evaluations of P-450 induction and TCDD-mediated cell proliferation by immunocytochemical methods in rat liver reveal that cells expressing CYP1A1 and 1A2 are different from those exhibiting TCDD-mediated increases in DNA replication (Lucier et al., 1992).

Placentas from Taiwanese women exposed to rice oil contaminated with polychlorinated dibenzofurans have markedly elevated levels of CYP1A1 (Lucier et al., 1987; Wong et al., 1986). Comparison of these data with induction data in rat liver suggests that humans are at least as sensitive as rats to the enzyme-inductive actions of TCDD and its structural analogs (Lucier, 1991). Consistent with this contention, the in vitro EC_{50} for TCDD-mediated induction of CYP1A1-dependent enzyme activities is approximately 1.5 nM when using either rodent or human lymphocytes (Clark et al., 1992). Also, binding of TCDD to the Ah receptor occurs with a higher affinity in rat cellular preparations compared with humans

(Lorenzen and Okey, 1991; Okey et al., 1989). This difference may be related to the greater lability of the human receptor during tissue preparation and cell fractionation procedures or to an inherent property of the human Ah receptor (Manchester et al., 1987). In any event, it does appear that humans contain a fully functional Ah receptor (Cook and Greenlee, 1989), as evidenced by significant CYP1A1 induction in tissues from exposed humans, and this response occurs with sensitivity similar to that observed in experimental animals.

6.5.2. Epidermal Growth Factor Receptor

EGF is a potent mitogen and stimulates the generation of mitotic signals in both normal and neoplastic cells (Stoscheck and King, 1986; Carpenter and Cohen, 1979). Several lines of evidence suggest that the EGF receptor and its ligands, including transforming growth factor-α, possess diverse functions relevant to cell transformation and tumorigenesis (Velu, 1990; Marti et al., 1989; Mukku and Stancel, 1985). In fact, the mechanism of action for several tumor promoters such as phenobarbital and the phorbol esters is thought to involve the EGF receptor pathway (Stoscheck and King, 1986). A schematic representation of the proposed mechanism for EGF-stimulated mitogenesis is given in Figure 6-3.

Several studies have shown that TCDD decreases the binding capacity of the plasma membrane EGF receptor for its ligand without a change in K_d (Clark et al., 1991a; Lin et al., 1991a; Abbott and Birnbaum, 1990; Astroff et al., 1990; Sunahara et al., 1989; Hudson et al., 1985; Madhukar et al., 1984). One study used a range of TCDD doses (3.5 to 125 ng/kg/day) for 30 weeks to evaluate the effects of TCDD exposure on EGF receptor in rat liver plasma membranes. There was a clear dose-response relationship for TCDD's effects on the total binding capacity of the EGF receptor although TCDD did not produce a change in binding affinity of the receptor. The maximal effect was a threefold decrease in the concentration of plasma membrane EGF receptor and the ED_{50} was ~ 10 ng/kg/day based on administered dose and ~ 2 ppb TCDD based on liver TCDD concentration. These values are similar to the ED_{50} for induction of CYP1A1 and CYP1A2 for 30-week exposures. The dose-response data, like the data for CYP1A1 and CYP1A2 induction, were subjected to curve-fitting analyses using the Hill equation (Portier et al., 1992). This analysis indicated that a Hill coefficient of 1 provided the best fit, suggesting that there is a linear relationship

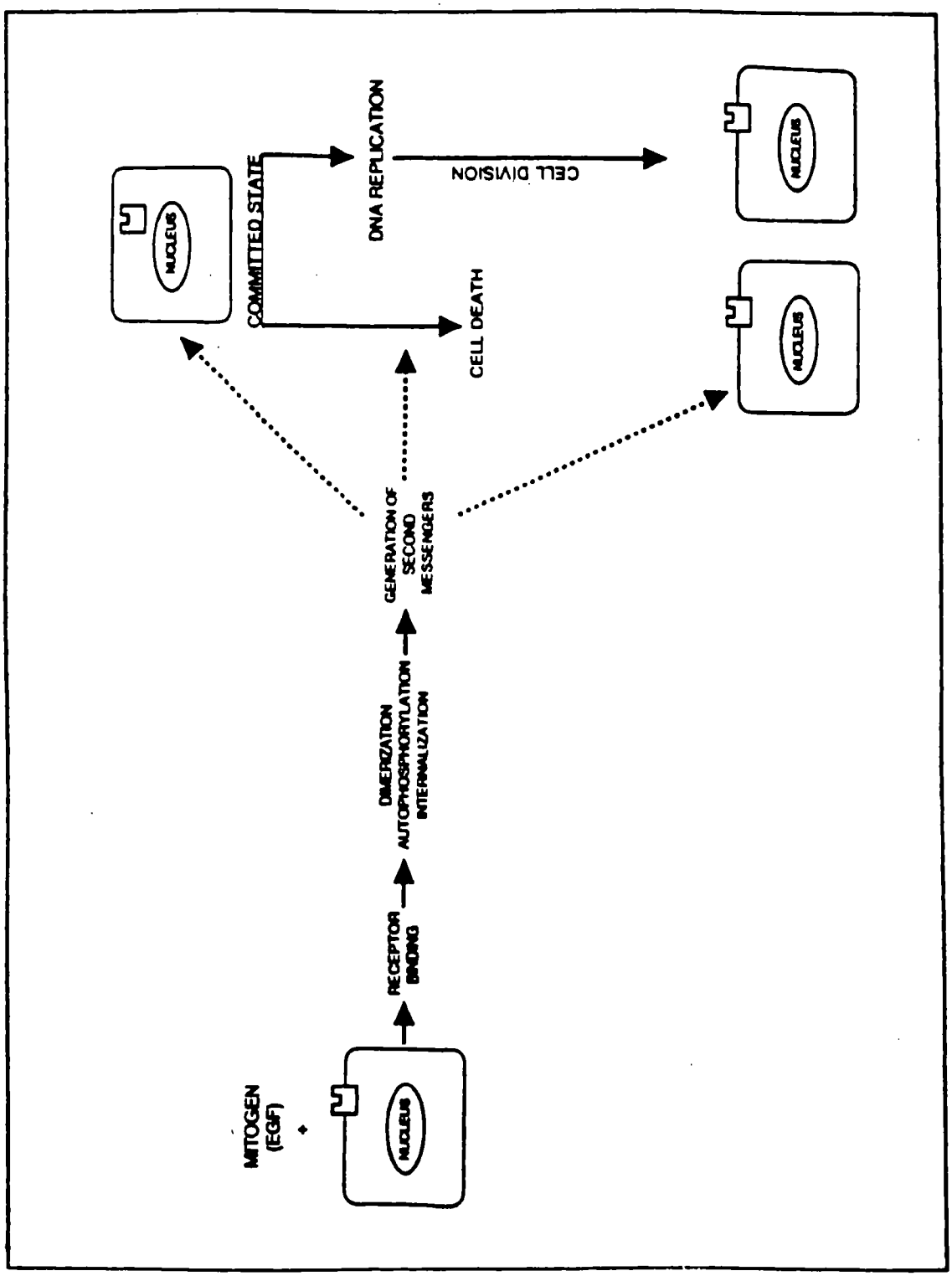

Figure 6-3. Plausible mechanism for the role of EGF-mediated stimulation of mitotic activity.

Source: Stoscheck and King, 1986.

between target tissue dose and the magnitude of response for effects on the EGF receptor. Although Hill analyses of dose-response data for TCDD's effects on the EGF receptor, CYP1A1 induction, and CYP1A2 induction are inconsistent with the idea of a threshold, the lowest dose used in these experiments was 100 pg/kg/day so the possibility exists that dose-response relationships are different in the very low-dose region (1 to 10 pg/kg/day) encountered as background human exposures.

Dose-response data on EGFR were compared with dose-response relationships for TCDD-mediated increases in cell proliferation and growth of preneoplastic lesions within the framework of a two-stage model for hepatocarcinogenesis in rats (Lucier et al., 1992). Results indicate that cell proliferation and the growth of preneoplastic lesions are less sensitive responses to TCDD than is loss of plasma membrane EGF receptor. Therefore, the EGF receptor may be involved in the hepatocarcinogenic actions of TCDD, but dose-response relationships for this effect may be different from dose-response relationships for liver cancer in rats. These data reflect the knowledge that several steps and/or several genes are involved in the modulation of coordinated biological responses.

The mechanism by which TCDD alters EGF receptor-binding capacity is not fully understood although TCDD does not appear to decrease EGF receptor mRNA (Lin et al., 1991a; Osborne et al., 1988). By using congenic mice deficient in the high-affinity Ah receptor, TCDD's effects on the EGF receptor were shown to require the Ah receptor (Lin et al., 1991a). In control animals, the EGF receptor is distributed on the surface of the plasma membrane and is composed of an external ligand-binding domain, a transmembrane domain, and an intercellular domain (Velu, 1990; Carpenter, 1987). Ligands for the EGF receptor (EGF or TGF-α) in the intracellular space bind the EGF receptor, producing a conformational change that stimulates the intercellular region to catalyze phosphorylation of the receptor itself as well as other proteins involved in cell regulation. The process results in internalization of the receptor characterized by an increase in cytosolic EGFR coupled with a decrease in membrane-bound receptor. The effects of TCDD and CDFs on the number of binding sites for the plasma membrane EGF receptor are correlated with a concomitant decrease in EGF-stimulated autophosphorylation of the EGF receptor, indicating that TCDD produces a true functional change in the EGF receptor (Clark et al., 1991a; Sunahara et al.,

1989; Nelson et al., 1988; Sunahara et al., 1988). Importantly, the addition of EGF to hepatocytes or several cell lines in culture produces a loss of plasma membrane EGF receptor coupled with a loss of EGF-stimulated autophosphorylation (Velu, 1990; Carpenter, 1987). Therefore, TCDD produces an EGF receptor-like response consistent with the idea that TCDD enhances the generation of cellular mitotic signals.

Although TCDD exposure mimics EGF actions in hepatocytes, TCDD itself does not appear to bind to the EGF receptor. The most plausible mechanism for effects on the EGF receptor involves the finding that TCDD induces production of TGF-α in hepatocytes as well as human keratinocytes (Choi et al., 1991). This response could alter control of normal growth patterns since TGF-α binds the EGF receptor with high affinity, leading to enhanced production of mitogenic signals. Alternatively, TCDD may affect EGF receptor transcription. In fact, TCDD has been shown to decrease uterine EGF receptor mRNA levels (Astroff et al., 1990). Receptor concentrations may also be altered by other events such as posttranslational glycosolation, increased lysosomal degradation, or alterations in signal transduction pathways such as protein kinases (Madhukar et al., 1988). It is also possible that TCDD alters phosphorylation of the EGF receptor by activation of protein kinase C, resulting in decreased binding capacity of the plasma membrane EGF receptor. This effect occurs following exposure to the tumor promoter TPA and is associated with decreased autophosphorylation rates and EGF receptor internalization (Beguinot et al., 1985; Cochet et al., 1984). In any event, TCDD-mediated alterations in EGF receptor pathways may, in part, be responsible for the tumor-promoting actions of TCDD by enhancement of mitotic signals.

The effects on the EGF receptor system may be mediated by estrogen action, and it has been postulated that the estrogen and EGF receptor pathways are integrated by "cross talk" mechanisms (Ignar-Trowbridge et al., 1992; Astroff et al., 1990). In vivo and in vitro studies have demonstrated that TCDD alters the estrogen receptor (DeVito et al., 1992; Lin et al., 1991a; Clark et al., 1991a; Umbreit and Gallo, 1988; Romkes et al., 1987) and estrogens can, in turn, alter EGF receptor binding and cellular distribution (Vickers and Lucier, 1991; Vickers et al., 1989; Mukku and Stancel, 1985). Moreover, studies conducted within the framework of a two-stage model for hepatocarcinogenesis have demonstrated that

TCDD-mediated decreases in plasma membrane EGF receptor are ovarian dependent (Clark et al., 1991a). These studies concluded that ovarian hormones are essential to the tumor-promoting actions of TCDD in that TCDD does not induce hepatocyte proliferation or stimulate the growth of preneoplastic lesions in ovariectomized rats (see Section 6.4, Initiation/Promotion Studies).

Evidence indicates that TCDD and its structural analogs produce the same effects on the EGF receptor in human cells and tissues as observed in experimental animals. First, incubation of human keratinocytes with TCDD decreases plasma membrane EGF receptor, and this effect is associated with increased synthesis of TGF-α (Choi et al., 1991; Hudson et al., 1985). Second, placentas from humans exposed to rice oil contaminated with polychlorinated dibenzofurans exhibit markedly reduced EGF-stimulated autophosphorylation of the EGF receptor, and this effect occurred with similar sensitivity to that observed in rats (Lucier, 1991; Sunahara et al., 1989). The magnitude of the effect on autophosphorylation was positively correlated with decreased birth weight of the offspring.

6.5.3. UDP-Glucuronosyltransferases

Several studies have shown that TCDD induces synthesis of at least one isozyme of UDPGT (Lucier et al., 1973, 1974, 1986) by a mechanism that requires the Ah receptor (Bock, 1991). The gene UGT-1 regulates synthesis of the UDPGT isozyme, which conjugates numerous substrates including 1-naphthol, p-nitrophenol, and thyroxine (Burchell et al., 1991). This gene contains a TCDD-responsive element that permits transcriptional activation following binding of the TCDD-Ah receptor complex. Other chemicals that bind the Ah receptor, such as 3-methylcholanthrene and benzo(a)pyrene, also induce UGT-1 (Bock, 1991). UDPGTs are considered a deactivation pathway for numerous environmental chemicals and endogenous compounds such as steroid hormones by rendering them water soluble and excretable as a consequence of the catalytic addition of a glucuronide moiety (Tephly and Burchell, 1990). Therefore, induction of UDPGT may be responsible, in part, for the finding that pretreatment with TCDD leads to diminished DNA adducts for PAHs and decreased concentrations of some steroid hormones.

Conjugation of thyroxine by UGT-1 leads to deactivation and elimination of this thyroid hormone (Henry and Gasiewicz, 1987; Bastomsky, 1977). The decreased levels of thyroxine, associated with UDPGT induction, produces decreased feedback inhibition of the pituitary gland, which responds by secreting increased amounts of TSH (Sanders et al., 1988; Barter and Klaassen, 1992). Several studies have provided evidence that prolonged stimulation by TSH produces an oncogenic effect on the thyroid (Hill et al., 1989). Interestingly, rat liver EGF receptor may, in part, be regulated by thyroid hormones (Mukku, 1984). Increased incidence of thyroid tumors is the most sensitive end point in cancer bioassays, as evidenced by a statistically significant increase at a dose of 1.4 ng/kg/day. Consistent with this hypothesis, rodent studies have shown that TCDD and other inducers of hepatic UDPGT decrease thyroxine concentration in blood, which is associated with increased levels of thyroid-stimulating hormone (Barter and Klaassen, 1992; Henry and Gasiewicz, 1987).

Dose-response studies for TCDD's inductive effects on hepatic UDPGT in rats have demonstrated that the single dose ED_{50} is approximately 0.7 μg/kg, which is similar to the ED_{50} for CYP1A1 induction (Lucier et al., 1986). Furthermore, the shape of the dose-response curve for both responses is similar. There are no data on UDPGT induction in long-term studies. Because humans have the dioxin-responsive UDPGT (UGT-1) (Burchell et al., 1991) and TCDD induces UDPGT in human hepatocyte cell cultures, it is reasonable to assume that TCDD and its structural analogs would induce UDPGT in humans although laboratory data are needed to validate this assumption.

6.5.4. Estrogen Receptor

Several lines of evidence have demonstrated that interactions of TCDD and estrogens are critical to some of the carcinogenic responses to TCDD. Although the precise mechanisms of those interactions have not been established, recent data indicate that TCDD effects on the ER and on estrogen metabolism are involved. The mechanisms for TCDD/estrogen interactions appear to be tissue specific. Of particular interest is the finding that TCDD increases liver tumor incidence in rats and at the same time decreases tumor incidence in organs such as the mammary gland, uterus, and pituitary (Kociba et al., 1978).

Therefore, TCDD/estrogen interactions will be examined separately for liver and other endocrine organs.

The liver contains a fully functional ER that possesses characteristics similar to those identified for ER in the mammary gland and uterus (Mastri and Lucier, 1983; Powell-Jones et al., 1981; Eisenfeld et al., 1976). For example, the liver exhibits high-affinity binding for 17β-estradiol and other potent estrogens, liver ER binding is specific for estrogens, the ligand receptor complex interacts reversibly with DNA, and this interaction leads to transcriptional activation of estrogen-responsive genes. Synthesis of hepatic ER, unlike ER in other target tissues, is under pituitary control (Lucier et al., 1981). Treatment of rats with a single dose of TCDD decreases binding capacity of the hepatic ER, and this effect is correlated with a decrease in ER protein (Zacharewski et al., 1991, 1992; Harris et al., 1990b; Romkes and Safe, 1988; Romkes et al., 1987). TCDD also decreases rat hepatic ER in chronic exposure experiments with a threefold decrease evident following a dose of 100 ng/kg/day for 30 weeks (Clark et al., 1991b). TCDD also decreases hepatic ER binding in C57Bl6 mice, but a much higher dose is needed to produce this effect in congenic mice deficient in the high-affinity Ah receptor, indicating that TCDD-mediated decreases in ER are dependent on the Ah receptor (Lin et al., 1991b). Dose-response studies in mice demonstrate that the single-dose ED_{50} is ~ 0.7 μg TCDD/kg, similar to the ED_{50} for other biochemical end points such as CYP1A1 induction, loss of plasma membrane EGF receptor, and induction of UDPGT. The observation that TCDD decreases hepatic ER is in apparent contradiction to the finding that TCDD increases hepatocyte proliferation because the ER is thought to produce mitogenic signals. However, quantitation of ER in control and TCDD-treated rats was done using preparations from liver homogenates. Immunolocalization studies are needed so that the relationship of ER concentrations to cell proliferation in normal and preneoplastic cells can be more carefully evaluated.

In addition to effects on hepatic ER, TCDD may influence estrogen action in another way. CYP1A2 efficiently catalyzes the conversion of estrogens to catechol estrogens in liver (Graham et al., 1988; Dannan et al., 1986). CYP1A2 is not found in extrahepatic tissues, with the possible exception of the nasal cavity, so catechol estrogen formation would be expected to occur only in liver. Catechol estrogens have been postulated to possess

macromolecule-damaging properties as a consequence of free radical generation (Li and Li, 1990; Metzler, 1984). Therefore, TCDD may increase the DNA-damaging capacity of estrogens in liver as a function of CYP1A2 induction. This effect may, in part, explain the carcinogenic actions of TCDD in female rat liver and is consistent with the knowledge that ovariectomy protects against the hepatocarcinogenic actions of TCDD and that male rats do not appear to be susceptible to TCDD-induced liver tumors (Lucier et al., 1991; Kociba et al., 1978). It is important to note that cancer is more than a two-stage process and the stage-specific actions of TCDD in multistage cancer models are not known, although TCDD-mediated cell proliferation and possible indirect genotoxic effects may be critical at more than one stage. A hypothetical mechanistic scheme for TCDD-mediated liver cancer is shown in Figure 6-2.

The finding that chronic TCDD exposure decreases tumor incidences in the pituitary, mammary gland, and uterus may also reflect TCDD's effects on ER and estrogen metabolism. As discussed above, TCDD decreases uterine ER concentrations in cytosolic and nuclear fractions of rats and mice, and these changes are associated with diminished estrogen action in in vivo as well as in vitro studies. TCDD also increases estrogen metabolism, presumably as a consequence of CYP1A2 in liver and UDPGT induction in liver and extrahepatic tissues (Shiverick and Muther, 1982). Likewise, the addition of TCDD to a breast cancer cell line (MCF-7) results in increased estrogen degradation (Gierthy et al., 1988). However, there are only small effects on serum 17-β estradiol levels following administration of TCDD to either rats or mice (Shiverick and Muther, 1983). Therefore, the effect on serum estradiol is considerably less sensitive than the effects on the uterine receptor. This comparison has led investigators to conclude that the antiestrogenic actions of dioxins are primarily caused by effects on ER levels in reproductive tract tissues. Consistent with this hypothesis, Fernandez and Safe (1992) have shown that TCDD is antimitogenic in human breast cancer cells. Final evaluation of the role of estrogen metabolism awaits data on concentrations of estrogens in responsive cells of control and TCDD-treated rats, which may be different from serum estradiol levels. In any event, it appears clear that TCDD does possess antiestrogenic properties that are likely to be important to decreased tumor incidences in some reproductive tract and endocrine organs. Numerous studies have documented that

the estrogen receptor is found in virtually every tissue of the body although the effects of TCDD on human estrogen receptor in vivo have not been studied.

6.5.5. Other Biochemical End Points

TCDD alters a number of other pathways involved in the regulation of cell differentiation and proliferation. The specific relationships of these effects to multistage carcinogenesis are not known, but the broad array of effects on hormone systems, growth factor pathways, cytokines, and signal transduction components is consistent with the notion that TCDD is a powerful growth dysregulator. It is also consistent with the findings that TCDD alters cancer risks at a large number of sites, possibly reflecting multiple mechanisms of carcinogenicity. Biochemical/molecular/endocrine changes produced by TCDD include the glucocorticoid receptor (Sunahara et al., 1989), tyrosine kinase (Madhukar et al., 1988), gastrin (Mabley et al., 1990), interleukin 1β (Sutter et al., 1991), plasminogen activator inhibitor (Sutter et al., 1991), tumor necrosis factor-α (Clark et al., 1991b), gonadotropin-releasing hormone (Moore et al., 1989), testosterone (Moore et al., 1985), and luteinizing hormone (Mabley et al., 1992). The importance of these responses to the carcinogenic process should not be diminished by the lack of detail presented here. In every case studied, these responses have been shown to be dependent on the Ah receptor.

6.6. SUMMARY AND WEIGHT OF EVIDENCE FROM ANIMAL STUDIES

There have been several long-term studies designed to determine if TCDD is a carcinogen in experimental animals. All of these studies have been positive and demonstrate that TCDD is a multisite carcinogen, is a carcinogen in both sexes and in several species including the Syrian hamster, is a carcinogen in sites remote from the site of treatment, and increases cancer incidence at doses well below the MTD. In two-stage models for liver and skin cancer, it is clear that TCDD is a potent promoting agent with weak or no initiating activity. This finding is not surprising because TCDD does not form DNA adducts and is negative in short-term tests for genetic toxicity. The general consensus is that TCDD is an example of receptor-mediated carcinogenesis in that (1) interaction with the Ah receptor appears to be a necessary early step, (2) TCDD modifies a number of receptor and hormone

systems involved in cell growth and differentiation such as the epidermal growth factor receptor and the estrogen receptor, and (3) hormones exert a profound influence on the carcinogenic actions of TCDD. For example, ovarian hormones are essential for the hepatocarcinogenic actions of TCDD in rats, whereas TCDD promotion of lung tumors in rats appears to occur only in the absence of ovarian hormones. Although tumor promotion data for the polychlorinated dibenzofurans and coplanar polychlorinated biphenyls are limited, it appears that these compounds are liver tumor promoters with potencies dependent on their binding affinity to the Ah receptor.

Some of the central issues in the risk assessment of TCDD and its structural analogs are (1) characterization of the shape of the dose-response curve for receptor-mediated events, (2) evaluation of the relevance of animal data in estimating human risks, and (3) the health consequences of background exposures (1 to 10 pg TEQ/kg/day) of dioxin and its structural analogs. With regard to the shape of the dose-response curve, it is clear from animal studies that there are different dose-response curves for different TCDD effects, which is consistent with the generally accepted dogma for steroid receptor-mediated responses. In general, the biochemical/molecular responses such as cytochrome P-450 induction do not show evidence for a threshold although unequivocal conclusions cannot be made about the mechanistic link, if any, between biochemical responses and toxic effects. In fact, coordinated biological responses such as TCDD-mediated cell proliferation and growth of preneoplastic lesions (foci of cellular alterations in liver) appear to be less sensitive end points, although evaluation of these responses is complicated by a high degree of interindividual variation: some animals do not exhibit any increase in cell proliferation in response to TCDD exposure.

The mechanistic basis for interindividual variation is unclear, and this lack of knowledge complicates approaches to estimate human risks from experimental animal data. However, several studies indicate that, for the most part, humans appear to respond like experimental animals for biochemical and carcinogenic effects. However, data from epidemiology studies are difficult to evaluate because the carcinogenic effects, if any, resulting from background TCDD exposures are not known, although biochemical effects such as cytochrome P-450 induction may be produced by background exposures.

REFERENCES FOR CHAPTER 6

Abbott, B.; Birnbaum, L. (1990) TCDD-induced altered expression of growth factors may have a role in producing cleft palate and enhancing the incidence of clefts after coadministration of retinoic acid and TCDD. Toxicol. Appl. Pharmacol. 106: 418-432.

Abernethy, D.J.; Greenlee, W.F.; Huband, J.C.; Boreiko, C.J. (1985) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) promotes the transformation of C3H/10T1/2 cells. Carcinogenesis 6: 651-653.

Abraham, K.; Krowke, R.; Neubert, D. (1988) Pharmacokinetics and biological activity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Arch. Toxicol. 62: 359-368.

Arcos, J.C.; Conney, A.H.; Buu-Hoi, N.P. (1961) Induction of microsomal enzyme synthesis by polycyclic aromatic hydrocarbons of different molecular sizes. J. Biol. Chem. 236: 1291.

Astroff, B.C.; Rowlands, R.; Dickerson, S.; Safe, S. (1990) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin inhibition of 17β-estradiol-induced increases in rat uterine epidermal growth factor receptor binding activity and gene expression. Mol. Cell. Endocrin. 70: 247-252.

Barrett, J.C. (1992) Multistage carcinogenesis. In: Vanio, H.; Magee, P.N.; McGregor, D.B.; McMichael, A.J., eds. Mechanisms of carcinogenesis in risk identification. Lyon, France: IARC, WHO.

Barrett, J.C.; Wiseman, R.W. (1987) Cellular and molecular mechanisms of multistep carcinogenesis: relevance to carcinogen risk assessment. Environ. Health Perspect. 76: 65-70.

Bars, R.G.; Elcombe, C.R. (1991) Dose-dependent acinar induction of cytochromes P450 in rat liver. Evidence for a differential mechanism of induction of P4501A1 by β-naphthaflavone and dioxin. Biochem. J. 277: 577-580.

Barter, R.A.; Klaassen, C.D. (1992) UDP-glucuronosyltransferase inducers reduce thyroid hormone levels in rats by an extrathyroidal mechanism. Toxicol. Appl. Pharmacol. 113: 36-42.

Bastomsky, C.H. (1977) Enhanced thyroxine metabolism and high uptake goiters in rats after a single dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Endocrinology 101: 292-296.

Beguinot, L.; Hanover, J.A.; Ito, S.; Richert, M.D.; Willingham, M.C.; Pastan, I. (1985) Phorbol esters induce internalization without degradation of unoccupied epidermal growth factor receptors. Proc. Natl. Acad. Sci. 82: 2774-2778.

Bock, K.W. (1991) Roles of UDP-glucuronyltransferases in chemical carcinogenesis. Crit. Rev. Biochem. Mol. Biol. 26(2): 129-150.

Burchell, B.; Nebert, D.W.; Nelson, D.R.; Bock, K.W.; Iyanagi, T.; Jansen, P.L.M.; Lancet, D.; Mulder, G.J.; Chowdhury, J.R.; Siest, G.; Tephly, T.R.; Mackenzie, P.I. (1991) The UDP glucuronlytransferase gene superfamily: suggested nomenclature based on evolutionary divergence. DNA Cell Biol. 10(7): 487-494.

California Department of Health Services. (1985) Health effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related compounds. Response to public comments. California Scientific Review Panel Discussions; September 1985.

Carpenter, G. (1987) Receptors for epidermal growth factor and other polypeptide mitogens. Ann. Rev. Biochem. 56: 881-914.

Carpenter, G.; Cohen, S. (1979) Epidermal growth factor. Ann. Rev. Biochem. 48: 193-216.

Choi, E.; Toscano, D.; Ryan, J.; Reidel, N.; Toscano, W. (1991) Dioxin induces transforming growth factor-α in human keratinocytes. J. Biol. Chem. 266: 9591-9597.

Clark, G.C.; Tritscher, A.; Maronpot, R.; Foley J.; Lucier, G. (1991a) Tumor promotion by TCDD in female rats. In: Gallo, M.; Scheuplein, R.; Van Der Heijden, K., eds. Banbury Report 35: biological basis for risk assessment of dioxin and related compounds. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; pp. 389-404.

Clark, G.C.; Taylor, M.J.; Tritscher, A.M.; Lucier, G.W. (1991b) Tumor necrosis factor involvement in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated endotoxin hypersensitivity in C57BL/6J mice congenic at the Ah locus. Toxicol. Appl. Pharmacol. 111: 422-431.

Clark, G.; Tritscher, A.; Bell, D.; Lucier, G. (1992) Integrative approach for evaluating species and interindividual differences in responsiveness to dioxins and structural analogs. Environ. Health Perspect. 98: 125-132.

Cochet, C.; Gill, G.N.; Meisenhelder, J.; Cooper, J.A.; Hunter, T. (1984) C-kinase phosphorylates the epidermal growth factor receptor and reduces its epidermal growth factor-stimulated tyrosine protein kinase activity. J. Biol. Chem. 259: 2553-2558.

Cohen, G.M.; Bracken, W.M.; Iyer, R.P.; Berry, D.L.; Selkirk, J.K.; Slaga, T.J. (1979) Anticarcinogenic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on benzo[a]pyrene and 7,12-dimethylbenz[a]anthrene tumor initiation and its relationship to DNA binding. Cancer Res. 39: 4027-4033.

Conney, A.H. (1982) Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons. G.H.A. Clowes Memorial Lecture. Cancer Res. 42: 4875-4917.

Cook, J.C.; Greenlee, W.F. (1989) Characterization of a specific binding protein for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in human thymic epithelial cells. Mol. Pharmacol. 35: 713-719.

Dannan, G.A.; Porubeck, D.J.; Nelson, S.D.; Waxman, D.J.; Guengerich, F.P. (1986) 17β-Estradiol 2- and 4-hydroxylation catalyzed by developmental patterns, and alterations in gonadectomy. Endocrinology 118: 1952-1960.

Della Porta, G.; Dragani, T.A.; Sozzi, G. (1987) Carcinogenic effects of infantile and long-term 2,3,7,8-tetrachlorodibenzo-*p*-dioxin treatment in the mouse. Tumori 73: 99-107.

DeVito, M.J.; Thomas, T.; Martin, E.; Umbreit, T.H.; Gallo, M.A. (1992) Antiestrogenic action of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: tissue specific regulation of estrogen receptor in CD1 mice. Toxicol. Appl. Pharmacol. 113: 284-292.

DeVito, M.J.; Umbreit, T.H.; Thomas, T.; Gallo, M.A. (1991) An analogy between the actions of the Ah receptor and the estrogen receptor for use in the biological basis for risk assessment of dioxin. In: Gallo, M.A.; Scheuplein, R.J.; Van Der Heijden, K.A., eds. Banbury Report 35: biological basis for risk assessment of dioxin and related compounds. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; pp. 427-440.

DiGiovanni, J.; Viaje, A.; Berry, D.L.; Slaga, T.; Juchau, M.R. (1977) Tumor initiating ability of TCDD and Arochlor 1254 in the two stage system of mouse skin carcinogenesis. Bull. Environ. Contam. Toxicol. 18: 552-557.

Dragan, Y.P.; Rizvi, T.; Xu, Y.H.; Holly, J.R.; Bawa, N.; Campbell, H.A.; Maronpot, R.R.; Pitot, H.C. (1991) An initiation-promotion assay in rat liver as a potential complement to the 2-year carcinogenesis bioassay. Fund. Appl. Toxicol. 16: 525-547.

Dragan, Y.P.; Xu, X.; Goldsworthy, T.L.; Campbell, H.A.; Maronpot, R.R.; Pitot, H.C. (1992) Characterization of the promotion of altered hepatic foci by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the female rat. Carcinogenesis 13(8): 1389-1395.

Eckl, P.M.; Meyer, S.A.; Whitcombe, W.R.; Jirtle, R.L. (1988) Phenobarbital reduces EGF receptors in the ability of physiological concentrations of calcium to suppress hepatocyte proliferation. Carcinogenesis 9: 479-483.

Eisenfeld, A.J.; Aten, R.; Weinberger, M.J.; Haselbacher, G. (1976) Estrogen receptor in the mammalian liver. Science 191: 862-865.

EPA Science Advisory Board. (1984) Environmental Health Committee Transcripts. November 29, 1984. Washington, DC: Science Advisory Board.

EPA Science Advisory Board. (1989) Review of draft documents: a cancer risk-specific dose estimate for 2,3,7,8-TCDD and estimating risk exposure to 2,3,7,8-TCDD. Washington, DC: EPA SAB Ad Hoc Dioxin Panel.

Farber, E. (1984) The multiple nature of cancer development. Cancer Res. 44: 4217-4223.

Fernandez, P.; Safe, S. (1992) Growth inhibitory and antimitogenic activity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in T47D human breast cancer cells. Toxicol. Lett. 61: 185-197.

Fingerhut, M.A.; Halperin, W.E.; Marlow, D.A.; Placitelli, L.A.; Honchar, P.A.; Sweeny, M.H.; Gireife, A.L.; Dill, P.A.; Steenland, K.; Suruda, A.J. (1991) Cancer mortality in workers exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. N. Engl. J. Med. 324: 212-218.

Flodstrom, S.; Ahlborg, U.G. (1991) Promotion of hepatocarcinogenesis in rats by PCDDs and PCDFs. In: Gallo, M.A.; Scheuplein, R.J.; Van Der Heijden, K.A., eds. Banbury Report 35: biological basis for risk assessment of dioxin and related compounds. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; pp. 405-414.

Flodstrom, S.; Ahlborg, U.G. (1992) Relative tumor promoting activity of some polychlorinated dibenzo-*p*-dioxin-, dibenzofuran-, and biphenyl congeners in female rats. Chemosphere 25:1(2): 169-172.

Gierthy, J.F.; Lincoln, D.W.; Kampick, S.J.; Dickerman, H.W.; Bradlow, H.L.; Niwa, T.; Swanek, G.E. (1988) Enhancement of 2- and 16-α-estradiol hydroxylation in MCF-7 human breast cancer cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Biochem. Biophys. Res. Commun. 157: 515-520.

Giri, A.K. (1987) Mutagenic and genotoxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: a review. Mutat. Res. 168: 241-248.

Goldstein, J.A.; Linko, P. (1984) Differential induction of two 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-inducible forms of cytochrome P-450 in extrahepatic versus hepatic tissues. Mol. Pharmacol. 25: 185-191.

Goldstein, J.A.; Safe, S. (1989) Mechanism of action and structure-activity relationships for the chlorinated dibenzo-*p*-dioxins and related compounds. In: Kimbrough, R.D.; Jensen, A.A., eds. Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins, and related products. New York: Elsevier; pp. 239-293.

Goodman, D.G.; Sauer, R.M. (1992) Hepatotoxicity in female Sprague-Dawley rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) pathology working group reevaluation. Regul. Toxicol. Pharmacol. 15: 245-252.

Graham, M.J.; Lucier, G.W.; Linko, P.; Maronpot, R.R.; Goldstein, J.A. (1988) Increases in cytochrome P-450 mediated 17β-estradiol 2-hydroxylase activity in rat liver microsomes after both acute administration and subchronic administration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a two-stage hepatocarcinogenesis model. Carcinogenesis 9(11): 1935-1941.

Greig, J.B.; DeMatteis, F. (1973) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on drug metabolism and hepatic microsomes of rats and mice. Environ. Health Perspect. 5: 211-220.

Guengerich, F.P. (1988) Roles of cytochrome P-450 enzymes in chemical carcinogenesis and cancer chemotherapy. Cancer Res. 48: 2946-2954.

Harris, M.; Zacharewski, T.; Piskorska-Pliszczynska, J.; Rosengren, R.; Safe, S. (1990a) Structure-dependent induction of aryl hydrocarbon hydroxylase activity in C57BL/6 mice by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related congeners: mechanistic studies. Toxicol. Appl. Pharmacol. 105: 243-253.

Harris, M.; Zacharewski, T.; Safe, S. (1990b) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related compounds on the occupied nuclear estrogen receptor in MCF-7 human breast cancer cell. Cancer Res. 50: 3579-3584.

Haseman, J.K.; Huff, J.E.; Boorman, G.A. (1984) Use of historical control data in carcinogenicity in rodents. Toxicol. Pathol. 12(2): 126-135.

Hayashi, K.; Sakamoto, N. (1986) Dynamic analysis of enzyme systems. Tokyo: Japan Scientific Societies Press.

Hebert, C.D.; Harris, M.W.; Elwell, M.R.; Birnbaum, L.S. (1990) Relative toxicity and tumor-promoting ability of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PCDF), and 1,2,3,4,7,8-hexachlorodibenzofuran (HCDF) in hairless mice. Toxicol. Appl. Pharmacol. 102: 362-377.

Henry, E.C.; Gasiewicz, T.A. (1987) Changes in thyroid hormones and thyroxine glucuronidation in hamsters compared with rats following treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. 89: 165-174.

Hill, R.N.; Erdreich, L.S.; Paynter, O.E.; Roberts, P.A.; Rosenthal, S.L.; Wilkinson, C.F. (1989) Thyroid follicular cell carcinogenesis. Fund. Appl. Toxicol. 12: 629-697.

Hudson, L.G.; Toscano, W.A., Jr.; Greenlee, W.F. (1985) Regulation of epidermal growth factor binding in human keratinocyte cell line by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. 77: 251-259.

Huff, J.E. (1992) 2,3,7,8-TCDD: a potent and complete carcinogen in experimental animals. Chemosphere 25: 173-176.

Huff, J.E.; Salmon, A.G.; Hooper, N.K.; Zeise, L. (1991) Long-term carcinogenesis studies on 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and hexachlorodibenzo-*p*-dioxins. Cell Biol. Toxicol. 7(1): 67-94.

Ignar-Trowbridge, D.M.; Nelson, K.G.; Bidwell, M.C.; Curtis, S.W.; Washburn, T.F.; McLachlan, J.A.; Korach, K.S. (1992) Coupling of dual signaling pathways: epidermal growth factor action involves the estrogen receptor. Proc. Natl. Acad. Sci. USA 89: 4658-4662.

IARC (International Agency for Research on Cancer). (1982) IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Suppl. 4: chemicals, industrial processes, and industries associated with cancer in humans. Lyon, France: WHO; pp. 238-243.

IARC (International Agency for Research on Cancer). (1992) Mechanisms of carcinogenesis in risk identification. Lyon, France: WHO.

Ito, N.; Tatematsu, M.; Nakanishi, K.; Hasegawa, R.; Takano, T.; Imaida, K.; Ogiso, T. (1980) The effects of various chemicals on the development of hyperplastic liver nodules in hepatectomized rats treated with N-nitrosodiethylamine or N-2-fluorenylacetamide. Jpn. J. Cancer Res. 71: 832-842.

Ito, N.; Tatematsu, M.; Hasegawa, R.; Tsuda, H. (1989) Medium-term bioassay system for detection of carcinogens and modifiers of hepatocarcinogenesis utilizing the GST-P positive liver cell focus as an endpoint marker. Toxicol. Pathol. 17(4 Part 1): 630-641.

Kedderis, L.B.; Diliberto, J.J.; Linko, P.; Goldstein, J.A.; Birnbaum, L.S. (1991) Disposition of 2,3,7,8-tetrabromodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat: biliary excretion and induction of cytochromes CYP1A1 and CYP1A2. Toxicol. Appl. Pharmacol. 111: 163-172.

Kitchin, K.T.; Woods, J.S. (1979) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) effects on hepatic microsomal cytochrome P-448-mediated enzyme activities. Toxicol. Appl. Pharmacol. 47: 537-546.

Kociba, R. (1984) Evaluation of the carcinogenic and mutagenic potential of 2,3,7,8-TCDD and other chlorinated dioxins. In: Poland, A.; Kimbrough, R., eds. Banbury Report 18: biological mechanisms of dioxin action. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; pp. 73-84.

Kociba, R.J.; Keyes, D.G.; Beyer, J.E.; Carreon, R.M.; Wade, D.A.; Dittenberger, D.A.; Kalnins, R.P.; Frauson, L.E.; Park, C.N.; Bernard, S.D.; Hummel, R.A.; Humiston, C.G. (1978) Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. Toxicol. Appl. Pharmacol. 46: 279-303.

Kohn, M.C.; Lucier, G.W.; Portier, C.J. (1993) A mechanistic model of effects of dioxin on gene expression in the rat liver. Fundam. Appl. Toxicol. 1: 48-56.

Kouri, R.E., Rude, T.H.; Joglekar, R.; Dansette, P.M.; Jerina, D.M.; Atlas, S.A.; Owens, I.S.; Nebert, D.W. (1978) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin as cocarcinogen causing 3-methylcholanthrene-initiated subcutaneous tumors in mice genetically "nonresponsive" at Ah locus. Cancer Res. 38: 2722-2783.

Levin, W.; Wood, A.; Chang, R.; Ryan, D.; Thomas, P.; Yagi, H.; Thakker, D.; Wyas, K.; Boyd, C.; Chu, S.Y.; Conney, A.; Jerina, D. (1982) Oxidative metabolism of polycyclic aromatic hydrocarbons to ultimate carcinogens. Drug Metab. Rev. 13: 555-580.

Li, J.J.; Li, S.A. (1990) Estrogen carcinogenesis in hamster tissues: a critical review. Endocr. Rev. 11(4): 524-531.

Lin, F.H.; Clark, G.; Birnbaum, L.S.; Lucier, G.W.; Goldstein, J.A. (1991a) Influence of the Ah locus on the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the hepatic epidermal growth factor receptor. Mol. Pharmacol. 39: 307-313.

Lin, F.; Stohs, S.; Birnbaum, L.; Clark, G.; Lucier, G.; Goldstein, J. (1991b) The effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the hepatic estrogen and glucocorticoid receptors in congenic strains of Ah responsive and Ah non-responsive C57BL/6J mice. Toxicol. Appl. Pharmacol. 108: 129-139.

Lorenzen, A.; Okey, A.B. (1991) Detection and characterization of Ah receptor in tissue and cells from human tonsils. Toxicol. Appl. Pharmacol. 107: 203-214.

Lucier, G.W. (1991) Humans are a sensitive species to some of the biochemical effects of structural analogs of dioxin. Environ. Toxicol. Chem. 10: 727-735.

Lucier, G.W. (1992) Receptor mediated carcinogenesis. In: Vanio, H.; Magee, P.N.; McGregor, D.B.; McMichael, A.J., eds. Mechanisms of carcinogenesis in risk identification. Lyon, France: IARC, WHO; pp. 87-112.

Lucier, G.W.; McDaniel, O.S.; Hook, G.E.R.; Fowler, B.; Sonawane, B.R.; Faeder, E. (1973) TCDD-induced changes in rat liver microsomal enzymes. Environ. Health Perspect. 5: 199-210.

Lucier, G.W.; McDaniel, O.S.; Hook, G.E.R. (1974) Nature of the enhancement of uridine diphosphate glucuronyltransferase activity by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rats. Biochem. Pharmacol. 24: 325-334.

Lucier, G.W.; Lui, E.M.K.; Lamartiniere, C.A. (1979) Metabolic activation/deactivation reactions during perinatal development. Environ. Health Perspect. 29: 7-16.

Lucier, G.W.; Slaughter, S.R.; Thompson, C.; Lamartiniere, C.A.; Powell-Jones, W. (1981) Selective actions of growth hormone on rat liver estrogen binding proteins. Biochem. Biophys. Res. Commun. 103: 872-879.

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

Lucier, G.W.; Nelson, K.G.; Everson, R.B.; Wong, T.K.; Philpot, R.M.; Tiernan, T.; Taylor, M.; Sunahara, G.I. (1987) Placental markers of human exposure to polychlorinated biphenyls and polychlorinated dibenzofurans. Environ. Health Perspect. 76: 79-87.

Lucier, G.W.; Tritscher, A.M.; Goldsworthy, T.; Foley, J.; Clark, G.; Goldstein, J.; Maronpot, R. (1991) Ovarian hormones enhance TCDD-mediated increases in cell proliferation and preneoplastic foci in a two stage model for hepatocarcinogenesis. Cancer Res. 51: 1391-1397.

Lucier, G.W.; Clark, G.; Tritscher, A.; Foley, J.; Maronpot, R. (1992) Mechanisms of dioxin tumor promotion: implications for risk assessment. Chemosphere 25(1-2): 177-180.

Lundgren, K.; Andries, M.; Thompson, C.; Lucier, G.W. (1986) Dioxin treatment of rats results in increased in vitro induction of sister chromatid exchanges by alpha-naphthoflavone: an animal model for human exposure to halogenated aromatics. Toxicol. Appl. Pharmacol. 85: 189-195.

Lundgren, K.; Andries, M.; Thomson, C.; Lucier, G.W. (1987) α-Naphthoflavone metabolized by 2,3,7,8-tetrachloro-*p*-dioxin induced rat liver microsomes: a potent clastogen in Chinese hamster ovary cells. Cancer Res. 47: 3662-3666.

Lundgren, K.; Collman, G.W.; Wang-Wuu, S.; Tiernan, T.; Taylor, M.; Thompson, C.L.; Lucier, G.W. (1988) Cytogenetic and chemical detection of human exposure to polyhalogenated aromatic hydrocarbons. Environ. Mol. Mutagen. 111: 1-11.

Mably, T.A.; Theobald, H.M.; Ingall, G.B.; Peterson, R.E. (1990) Hypergastrinemia is associated with decreased gastric acid secretion in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin treated rats. Toxicol. Appl. Pharmacol. 106: 518-528.

Mably, T.A.; Moore, R.W.; Goy, R.W.; Peterson, R.E. (1992) In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. 114: 108-117.

Madhukar, B.V.; Brewster, D.W.; Matsumura, F. (1984) Effects of in vivo administered 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on receptor binding of epidermal growth factor in the hepatic plasma membrane of rat, guinea pig, mouse and hamster. Proc. Natl. Acad. Sci. 81: 7407-7411.

Madhukar, B.V.; Ebner, K.; Matsumura, F.; Bombick, D.W.; Brewster, D.W.; Kawamoto, T. (1988) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin causes an increase in protein kinases associated with epidermal growth factor receptor in the hepatic plasma membrane. J. Biochem. Toxicol. 3: 261-277.

Manchester, D.K.; Gorson, S.K.; Golas, C.L.; Roberts, E.A.; Okey, A.B. (1987) Ah receptor in human placenta: stabilization by molybdate and characterization of binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 3-methylcholanthrene, and benzo[a]pyrene. Cancer Res. 47: 4861-4868.

Maronpot, R.R.; Montgomery, C.A.; Boorman, G.A.; McConnell, E.E. (1986) National Toxicology Program Nomenclature for hepatoproliferative lesions of rats. Toxicol. Pathol. 14(2): 263-273.

Maronpot, R.R.; Pitot, H.C.; Peraino, C. (1989) Use of rat liver altered focus models for testing chemicals that have completed two-year carcinogenicity studies. Toxicol. Pathol. 17(4 Part 1): 651-662.

Marti, M.; Burwen, S.; Jones, A. (1989) Biological effects of epidermal growth factor, with emphasis on the gastrointestinal tract and liver: an update. Hepatology 9: 126-139.

Mastri, C.; Lucier, G. (1983) Actions of hormonally active chemicals in the liver. In: Thomas, J.A., ed. Endocrine toxicology. New York, NY: Raven Press; pp. 335-355.

Metzler, M. (1984) Metabolism of stilbene estrogens and steroidal estrogens in relation to carcinogenicity. Arch. Toxicol. 55: 104-109.

Miller, E.C.; Miller, J.A.; Brown, R.R.; MacDonald, J.C. (1958) On the protective action of certain polycyclic aromatic hydrocarbons against carcinogenesis by aminoazo dyes and 2-acetylaminofluorene. Cancer Res. 18: 469.

Moolgavkar, S.; Knudson, A. (1981) Mutation and cancer: a model for human carcinogenesis. J. Natl. Cancer Inst. 66: 1037-1052.

Moore, R.W.; Parsons, J.A.; Bookstaff, R.C.; Peterson, R.E. (1989) Plasma concentrations of pituitary hormones in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated male rats. J. Biochem. Toxicol. 4: 165-172.

Moore, R.W.; Potter, C.L.; Theobald, H.M.; Robinson, J.A.; Peterson, R.E. (1985) Androgenic deficiency in male rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol. Appl. Pharmacol. 79: 99-111.

Mukku, V.R. (1984) Regulation of epidermal growth factor receptor levels by thyroid hormone. J. Biol. Chem. 259(10): 6543-6547.

Mukku, V.; Stancel, G. (1985) Regulation of epidermal growth factor receptor by estrogen. J. Biol. Chem. 260: 9820-9824.

NCI (National Cancer Institute). (1979a) Bioassay of dibenzo-*p*-dioxin for possible carcinogenicity. NCI Tech. Rept. No. 122. Bethesda, MD: National Institutes of Health.

NCI (National Cancer Institute). (1979b) Bioassay of dichlorodibenzo-*p*-dioxin for possible carcinogenicity. NCI Tech. Rept. No. 123. Bethesda, MD: National Institutes of Health.

NTP (National Toxicology Program). (1980) Bioassay of a mixture of 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin and 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin for possible carcinogenicity (gavage study). Tech. Rept. Ser. No. 198. Research Triangle Park, NC: U.S. DHHS, PHS.

NTP (National Toxicology Program). (1982a) Bioassay of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin for possible carcinogenicity (gavage study). Tech. Rept. Ser. No. 201. Research Triangle Park, NC: U.S. DHHS, PHS.

NTP (National Toxicology Program). (1982b) Bioassay of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin for possible carcinogenicity (dermal study). Tech. Rept. Ser. No. 201. Research Triangle Park, NC: U.S. DHHS, PHS.

NTP (National Toxicology Program). (1984) Report of the NTP Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation. Board of Scientific Counselors. Research Triangle Park, NC: U.S. DHHS, PHS.

Nelson, K.; Vickers, A.; Sunahara, G.; Lucier, G. (1988) Receptor and DNA ploidy changes during promotion of rat liver carcinogenesis. In: Langenbach, R.; Elmore, E.; Barrett, J., eds. Tumor promoters: biological approaches for mechanistic studies and assay systems. Progress in cancer research and therapy, v. 34. New York, NY: Raven Press; pp. 387-405.

Nemoto, N.; Gelboin, H.V. (1976) Enzymatic conjugation of benzo[a]pyrene oxides, phenols and dihydrodiols with UDP-glucuronic acid. Biochem. Pharmacol. 25: 1221-1226.

Okey, A.B.; Denison, M.S.; Prokipcak, R.D.; Roberts, E.A.; Harper, P.A. (1989) Receptors of polycyclic aromatic hydrocarbons. In: Galteau, M.M.; Siest, G.; Henny, J., eds. Biologie prospective. Paris: John Libbey Eurotext; pp. 605-610.

Osborne, R.; Cook, J.C.; Dold, K.M.; Ross, L.; Gaido, K.; Greenlee, W.F. (1988) TCDD receptor: mechanisms of altered growth regulation in normal and transformed human keratinocytes. In: Langenbach, R.; Burrett, J.C.; Elmore, E., eds. Progress in cancer research and therapy, v. 34. New York, NY: Raven Press; pp. 407-416.

Parkinson, A.; Hurwitz, A. (1991) Omeprazole and the induction of human cytochrome P-450: a response to concerns about potential adverse effects. Gastroenterology 100(4): 1157-1164.

Parkinson, A.; Thomas, P.E.; Ryan, D.E.; Reik, L.M.; Safe, S.H.; Robertson, L.W.; Levin, W. (1983) Differential time course of induction of rat liver microsomal cytochrome P450 isozymes and epoxide hydrolase by Arochlor 1254. Arch. Biochem. Biophys. 225: 203-215.

Pelkonnen, O.; Nebert, D.W. (1982) Metabolism of polycyclic aromatic hydrocarbons: etiologic role in carcinogenesis. Pharmacol. Rev. 34: 189-222.

Peraino, C., Staffeldt, E.F.; Ludeman, V.A. (1981) Early appearance of histochemically altered hepatocyte foci and liver tumors in female rats treated with carcinogens 1 day after birth. Carcinogenesis 2: 463-465.

Pitot, H.C.; Campbell, H.A. (1987) An approach to the determination of the relative potencies of chemical agents during the stages of initiation and promotion in multistage hepatocarcinogenesis in the rat. Environ. Health Perspect. 76: 49-56.

Pitot, H.C.; Sirica, A.E. (1980) The stages of initiation and promotion in hepatocarcinogenesis. Biochim. Biophys. Acta 605: 191-215.

Pitot, H.C.; Goldsworthy, T.L.; Campbell, H.A.; Poland, A. (1980) Quantitative evaluation of the promotion by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin of hepatocarcinogenesis from diethylnitrosamine. Cancer Res. 40: 3616-3620.

Pitot, H.C.; Goldsworthy, T.L.; Moran, S.; Kennan, W.; Glauert, H.P.; Maronpot, R.R.; Campbell, H.A. (1987) A method to quantitate the relative initiating and promoting potencies of hepatocarcinogenic agents in their dose-response relationships to altered hepatic foci. Carcinogenesis 8: 1491-1499.

Pitot, H.C.; Campbell, H.A.; Maronpot, R.R.; Bawa, N.; Rizvi, T.A.; Xu, Y.; Sargent, L.; Dragan, Y.; Pyron, M. (1989) Critical parameters in the quantitation of the stages of initiation, promotion, and progression in one model of hepatocarcinogenesis in the rat. Toxicol. Pathol. 17(4 Part 1): 594-612.

Poland, A.; Glover, E. (1973) Studies on the mechanism of toxicity of chlorinated dibenzo-*p*-dioxins. Environ. Health Perspect. 5: 245-252.

Poland, A.; Knutson, J.C. (1982) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: examination of the mechanism of toxicity. Ann. Rev. Pharmacol. Toxicol. 22: 517-554.

Poland, A.; Palen, D.; Glover, E. (1982) Tumor promotion by TCDD in skin of HRS/J mice. Nature 300(5889): 271-273.

Popp, J.A.; Goldsworthy, T.L. (1989) Defining foci of cellular alteration in short-term and medium-term rat liver tumor models. Toxicol. Pathol. 17(4 Part 1): 561-568.

Portier, C.J. (1987) Statistical properties of a two-stage model of carcinogenesis. Environ. Health Perspect. 76: 125-132.

Portier, C.; Tritscher, A.; Kohn, M.; Sewall, C.; Clark, G.; Edler, L.; Hoel, D.; Lucier, G. (1993) Ligand/receptor binding for 2,3,7,8-TCDD: implications for risk assessment. Fund. Appl. Toxicol. 20(1): 48-56.

Pour, P.; Kmoch, N.; Greiser, E.; Mohr, U.; Althoff, J.; Cardesa, A. (1976) Spontaneous tumors and common diseases in two colonies of Syrian hamsters: I. Incidence and sites. J. Natl. Cancer Inst. 56(5): 931-935.

Powell-Jones, W.; Thompson, C.; Raeford, S.; Lucier, G.W. (1981) Effect of gonadectomy on the ontogeny of estrogen-binding components in the rat liver cytosol. Endocrinology 109: 628-636.

Randerath, K.; Putman, K.L.; Randerath, E.; Mason, G.; Kelly, M.; Safe, S. (1988) Organ-specific effects of long-term feeding of 2,3,7,8-tetrachlorodibenzo-p-dioxin and 1,2,3,7,8-pentachlorodibenzo-p-dioxin on I-compounds in hepatic and renal DNA of female Sprague-Dawley rats. Carcinogenesis 9(12): 2285-2289.

Rao, M.S.; Subbarao, V.; Prasad, J.D.; Scarpelli, D.C. (1988) Carcinogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the Syrian golden hamster. Carcinogenesis 9(9): 1677-1679.

Roberts, L. (1991) Dioxin risks revisited. Science 251: 624-626.

Romkes, M.; Safe, S. (1988) Comparative activities of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and progesterone on antiestrogens in the female rat uterus. Toxicol. Appl. Pharmacol. 92: 368-380.

Romkes, M.; Piskorska-Pliszczynska, J.; Safe, S. (1987) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on hepatic and uterine estrogen receptor levels in rats. Toxicol. Appl. Pharmacol. 87: 306-314.

Sanders, J.E.; Eigenberg, D.A.; Bracht, L.J.; Wang, W.R.; Van Zwieten, J.J. (1988) Thyroid and liver trophic changes in rats secondary to liver microsomal enzyme induction caused by an experimental leukotriene antagonist (L-649,923). Toxicol. Appl. Pharmacol. 95: 378-387.

Sauer, R.M. (1990) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in Sprague-Dawley rats. Submitted to the Maine Scientific Advisory Panel by Pathco, Inc., Ijamsville, MD. March 13, 1990.

Shi, Y.E.; Yager, J.D. (1989) Effects of the liver tumor promoter ethinyl estradiol on epidermal growth factor-induced DNA synthesis and epidermal growth factor receptor levels in cultured rat hepatocytes. Cancer Res. 49: 3574-3580.

Shiverick, K.T.; Muther, T.F. (1982) Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on serum concentrations and the uterotrophic actions of exogenous estrone in rats. Toxicol. Appl. Pharmacol. 65: 170-176.

Shiverick, K.T.; Muther, T.F. (1983) 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) effects on hepatic microsomal steroid metabolism and serum estradiol of pregnant rats. Biochem. Pharmacol. 32: 991-995.

Shu, H.P.; Paustenbach, D.J.; Murray, F.J. (1987) A critical evaluation of the use of mutagenesis, carcinogenesis and tumor promotion data in a cancer risk assessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Reg. Toxicol. Pharmacol. 7: 57-58.

Silbergeld, E.K.; Gasiewicz, T.A. (1989) Dioxins and the Ah receptor. Am. J. Ind. Med. 16: 455-474.

Sims, P.; Glover, P.L. (1974) Epoxides in polycyclic aromatic hydrocarbon metabolism and carcinogenesis. Adv. Cancer Res. 20: 165-274.

Slaga, T.J.; Becker, L.; Bracken, W.M.; Weeks, C.E. (1979) The effects of weak or non-carcinogenic polycyclic hydrocarbons on 7,12-dimethyl benz[a]anthracene and benzo[a]pyrene skin tumor initiation. Cancer Lett. 7: 51-59.

Slaga, T.J.; Fischer, S.M.; Weeks, C.E.; Klein-Szanto, A.J.P.; Reiners, J. (1982) Studies on the mechanisms involved in multistage carcinogenesis in mouse skin. J. Cell. Biochem. 18: 99-119.

Sloop, T.C.; Lucier, G.W. (1987) Dose-dependent elevation of Ah receptor binding by TCDD in rat liver. Toxicol. Appl. Pharmacol. 88: 329-337.

Squire, R.A. (1980) Pathologic evaluations of selected tissues from the Dow chemical TCDD and 2,4,5-T rat studies. Submitted to Carcinogen Assessment Group, U.S. Environmental Protection Agency on August 15 under contract no. 68-01-5092.

Stoscheck, C.; King, L. (1986) Role of epidermal growth factor in carcinogenesis. Cancer Res. 46: 1030-1037.

Sunahara, G.I.; Nelson, K.G.; Wong, T.K.; Lucier, G.W. (1988) Decreased human birth weights after in utero exposure to PCBs and PCDFs are associated with decreased placental EGF-stimulated receptor autophosphorylation capacity. Mol. Pharmacol. 32: 572-578.

Sunahara, G.; Lucier, G.; McCoy, Z.; Bresnick, E.; Sanchez, E.; Nelson, K. (1989) Characterization of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated decreases in dexamethasone binding to rat hepatic cytosolic glucocorticoid receptor. Mol. Pharmacol. 36: 239-247.

Sutter, T.R.; Greenlee, W.F. (1992) Classification of members of the Ah gene battery. Chemosphere 25: 223-226.

Sutter, T.R.; Guzman, K.; Dold, K.M.; Greenlee, W.F. (1991) Targets for dioxin: genes for plasminogen activator inhibitor-2 and interleukin-1β. Science 254: 415-417.

Swenberg, J.A.; Richardson, F.C.; Baucheron, J.A.; Deal, F.H.; Belinsky, S.A.; Charbonneau, M.; Short, B.G. (1987) High- to low-dose extrapolation: critical determinants involved in the dose response of carcinogenic substances. Environ. Health Perspect. 76: 57-64.

Tephly, T.R.; Burchell, B. (1990) UDP-glucuronyltransferases: a family of detoxifying enzymes. TIPS Rev. 11: 276-279.

Thakker, D.R.; Yagi, H.; Levin, W.; Wood, A.W.; Conney, A.H.; Jerina, D.M. (1985) Polycyclic aromatic hydrocarbons: metabolic activation to ultimate carcinogens. In: Anders, M.W., ed. Bioactivation of foreign compounds. New York, NY: Academic Press; pp. 177-242.

Tritscher, A.M.; Goldstein, J.A.; Portier, C.J.; McCoy, Z.; Clark, G.C.; Lucier, G.W. (1992) Dose-response relationships for chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in a rat tumor promotion model: quantification and immunolocalization of CYP1A1 and CYP1A2 in the liver. Cancer Res. 52: 3436-3442.

Turteltaub, K.W.; Felton, J.S.; Gledhill, B.L.; Vogel, J.S.; Southon, J.R.; Caffee, M.W.; Finkel, R.C.; Nelson, D.E.; Procter, I.D.; David, J.C. (1990) Accelerator mass spectrometry in biomedical dosimetry: relationship between low-level exposure and covalent binding of heterocyclic amine carcinogens to DNA. Proc. Natl. Acad. Sci. USA 87: 5288-5292.

Umbreit, T.H.; Gallo, M.A. (1988) Physiological implications of estrogen receptor modulation by 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin. Toxicol. Lett. 42: 5-14.

U.S. EPA (1985) Health effects assessment document for polychlorinated dibenzo-*p*-dioxins. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH, for the Office of Emergency and Remedial Response, Washington, DC. EPA 600/8-84-014F.

Vanden Heuvel, J.P.; Lucier, G.W. (1993) Environmental toxicology of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans. Environ. Health Perspect. 100: 189-200.

Vecchi, A.; Sironi, M.; Canegrati, M.A.; Recchia, M.; Garatini, S. (1983) Immunosuppressive effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in strains of mice with different susceptibility to induction of aryl hydrocarbon hydroxylase. Toxicol. Appl. Pharmacol. 68: 434-441.

Velu, T. (1990) Structure, function and transforming potential of the epidermal growth factor receptor. Mol. Cell. Endocrin. 70: 205-216.

Vickers, A.; Lucier, G. (1991) Estrogen receptor, epidermal growth factor and cellular ploidy in elutriated subfractions of hepatocytes during liver promotion by 17α-ethinylestradiol in rats. Carcinogenesis 12: 391-399.

Vickers, A.E.M.; Nelson, K.; McCoy, Z.; Lucier, G.W. (1989) Changes in estrogen receptor, DNA ploidy, and estrogen metabolism in rat hepatocytes during a two-stage model for hepatocarcinogenesis using 17-ethinylestradiol as a promoting agent. Cancer Res. 49: 6512-6520.

Wahba, Z.Z.; Lawson, T.A.; Stohs, S.J. (1988) Induction of hepatic DNA single strand breaks in rats by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Cancer Lett. 29: 281-286.

Wahba, Z.Z.; Lawson, T.A.; Murray, W.J.; Stohs, S.J. (1989) Factors influencing the induction of DNA single strand breaks in rats by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Toxicology 58: 57-69.

Wassom, J.S.; Huff, J.E.; Loprieno, N. (1977) A review of the genetic toxicology of chlorinated dibenzo-*p*-dioxins. Mutat. Res. 47: 141-160.

Wattenberg, L.W. (1978) Inhibition of chemical carcinogenesis. J. Natl. Cancer Inst. 60: 11-18.

Wattenberg, L.W. (1985) Chemoprevention of cancer. Cancer Res. 45: 108.

Wattenberg, L.W.; Leong, J.L. (1970) Inhibition of the carcinogenic action of benzo(a)pyrene by flavones. Cancer Res. 30: 1922-1925.

Wheatley, D.N. (1968) Enhancement and inhibition of the induction by 7,12-dimethylbenz(a)anthracene of mammary tumors in female Sprague-Dawley rats. Br. J. Cancer 22: 787-797.

Whitlock, J.P., Jr. (1990) Genetic and molecular aspects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin action. Ann. Rev. Pharmacol. Toxicol. 30: 251-277.

Williams, G.M. (1989) The significance of chemically-induced hepatocellular altered foci in rat liver and application to carcinogen detection. Toxicol. Pathol. 17(4 Part 1): 663-674.

Wong, T.K.; Domin, B.A.; Bent, P.E.; Blanto, T.E.; Anderson, M.W.; Philpot, R.M. (1986) Correlation of placental microsomal activities with protein detected by antibodies to rabbit cytochrome P-450 isozyme 6 in preparations from humans exposed to polychlorinated biphenyls, quarterphenyls, and dibenzofurans. Cancer Res. 46: 999-1004.

Yang, Jae-Ho; Thraves, P.; Dritschilo, A.; Rhim, J.S. (1992) Neoplastic transformation of immortalized human keratinocytes by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Cancer Res. 52: 3478-3482.

Zacharewski, T.; Harris, M.; Safe, S. (1991) Evidence for a possible mechanism of action of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated decrease of nuclear estrogen receptor levels in wild-type and mutant Hepa 1c1c7 cells. Biochem. Pharmacol. 41: 1931-1939.

Zacharewski, T.; Harris, M.; Biegel, L.; Morrison, V.; Merchant, M.; Safe, S. (1992) 6-Methyl-1,3,8-trichlorodibenzofuran (MCDF) as an antiestrogen in human and rodent cancer cell lines: evidence for the role of the Ah receptor. Toxicol. Appl. Pharmacol. 113: 311-318.

Zeise, L.; Huff, J.E.; Salmon, A.G.; Hooper, N.K. (1990) Human risks from 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and hexachlorodibenzo-*p*-dioxins. In: Advances in modern environmental toxicology, v. 17. Princeton, NJ: Princeton Scientific Publishing Co., Inc.; pp. 293-342.