Developmental and Reproductive Toxicity of Dioxins and Related Compounds: Cross-Species Comparisons

Richard E. Peterson, Robert J. Theobald, and Gary L. Kimmel

*School of Pharmacy and Environmental Toxicology Center, University of Wisconsin, Madison, WI; and U.S. Environmental Protection Agency, Washington, D.C.

ABSTRACT: Developmental toxicity to TCDD-like congeners in fish, birds, and mammals, and reproductive toxicity in mammals are reviewed. In fish and bird species, the developmental lesions observed are species dependent, but any given species responds similarly to different TCDD-like congeners. Developmental toxicity in fish resembles "blue-sac disease," whereas structural malformations can occur in a number of bird species. In mammals, developmental toxicity includes decreased growth, structural malformations, functional alterations, and prenatal mortality. At relatively low exposure levels, structural malformations are not common in all species. In contrast, functional alterations are the most sensitive signs of developmental toxicity. These include effects on the male reproductive system and male reproductive behavior in rats, and neurobehavioral effects in monkeys. Human infants exposed during the Yusho and Yu-Cheng episodes, and monkeys and mice exposed perinatally to TCDD developed an ectodermal dysplasia syndrome that includes toxicity to the skin and teeth. Toxicity to the central nervous system in monkey and human infants is a potential part of the ectodermal dysplasia syndrome. Decreases in spermatogenesis and the ability to conceive and carry a pregnancy to term are the most sensitive signs of reproductive toxicity in male and female mammals, respectively.

KEY WORDS: perinatal exposure, structural malformations, functional alterations, antiestrogenicity, sexual differentiation, Ah receptor.

I. INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is one of 75 possible chlorinated dibenzo-p-dioxin (CDD) congeners. It is one of the most potent of the CDDs. Brominated dibenzo-p-dioxins (BDDs), chlorinated dibenzofurans (CDFs), brominated dibenzofurans (BDFs), polychlorinated biphenyls (PCBs), and polybrominated biphenyls (PBBs) all serve 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; 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.\textsuperscript{2,3} PCB congeners with zero or one ortho chlorine, 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.\textsuperscript{1,3}

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.\textsuperscript{4-6} Furthermore, coplanar PCBs and/or monoortho-chlorine-substituted analogs of the coplanar PCBs bioaccumulate in fish, wildlife, and humans.\textsuperscript{7-11} This is of concern because the combined effects of the lateral-substituted CDD, BDD, CDF, BDF, PCB, and PBB congeners that act through an aryl hydrocarbon (Ah) receptor mechanism have the potential to decrease feral fish and wildlife populations secondary to developmental and reproductive toxicity.\textsuperscript{5,12-14} 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 nonTCDD-like congeners have been implicated in causing the developmental and reproductive toxicity in the Yusho and Yu-Cheng poisoning incidents in Japan and Taiwan.\textsuperscript{15-17} Thus, exposure to TCDD-like congeners is a health concern for humans, domesticated animals, fish, and wildlife, although the relative contributions of TCDD- and nonTCDD-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-response elements (DREs), resulting in alterations in gene transcription. There is evidence that this Ah receptor mechanism is involved in the antiestrogenic action of TCDD and in its ability to produce the structural malformations cleft palate and hydronephrosis in mice. However, its role in producing other signs of developmental and reproductive toxicity is less firmly established or not established. This leaves open the possibility for some of these TCDD effects to not be Ah receptor mediated.

The information in this review has been organized into sections on developmental toxicity, reproductive toxicity in males, and reproductive toxicity in females. However, it is important to emphasize that developmental and reproductive toxicity, particularly in females, can be interrelated. Therefore, the reader should view the section subheadings as topic indexes that indicate where most of the information on a particular endpoint is concentrated. The endpoints described within each subheading are not intended to be thought of as being independent of endpoints described in other sections. For example, the effects of TCDD on the actions of reproductive hormones, peptides, and steroids can be involved in reproductive dysfunction as well as developmental toxicity.

II. DEVELOPMENTAL TOXICITY

The manifestations of developmental toxicity to TCDD have been divided into three categories for convenience in assessing the data base 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. However, in other mammalian species, 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 reproductive system of rats and object-learning behavior in monkeys.
## A. Death/Growth/Clinical Signs

### 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). 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. Cooper reviewed the developmental toxicity of CDDs and CDFs in fish and Cook et al. discussed components of an aquatic ecological risk assessment for TCDD in fish. The reader is referred to this literature for more in-depth 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. Histopathological evaluation of lake trout embryos and sac fry has shown this syndrome to be essentially identical to that of blue sac disease. Following egg exposure to TCDD, signs of toxicity are not detected in medaka until after the liver rudiment forms, and in lake trout, toxicity is first detected approximately 1 week prior to hatching, but becomes fully manifest during the sac fry stage. 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% mortality above control at swim up (LD_{50}) is 65 pg TCDD/g egg. 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

---

### DATE DUE

<table>
<thead>
<tr>
<th>NOV 15</th>
<th>15 Dec</th>
</tr>
</thead>
</table>

---

### E. Dinos

Bird embryos also are 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 to 50 μg/kg body weight). The LD_{50} of TCDD injected into fertilized ring-necked pheasant eggs (1.1 to 1.8 μg/kg egg weight) is 14 to 23 times less than the TCDD dose that causes 75% mortality in ring-necked hen pheasants (25 μg/kg body weight). Among bird species, most of the developmental toxicity research has been done on chickens. Injection of TCDD or its approximate isomers 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, microophthalmia, beak deformities, cardiovascular malformations, and mortality. On the other hand, injection of a coplanar PCB into fertilized turkey eggs at a dose high enough to cause microophthalmia, beak deformities, and embryo mortality did not
produce liver lesions, edema, or thymic hypoplasia, all hallmark signs of TCDD toxicity in the chicken embryo. Thus, 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. Thus, in bird embryos, the signs of toxicity elicited by TCDD and its approximate isomers 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 and the rank order potency of PCB congeners for producing chicken embryo mortality is 3,3',4,4',5-PCB > 3,3',4,4'-TCB > 3,3',4,4',5,5'-HCB > 2,3,3',4,4'-PCB > 2,3,3',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, and this rank order is similar to that for a classic Ah receptor-mediated response in the chicken embryo, i.e., cytochrome P-450IA1 induction. Inasmuch as the nonsteroidal anti-inflammatory drug benoxaprofen suppresses 3,3',4,4'-TCB-induced toxicity in the chicken embryo without altering its ability to induce microsomal enzyme activity, induction of cytochrome P-450IA1 and toxicity are part of a pleiotropic response linked to the Ah receptor, but these effects are not otherwise causally related. 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.

A decrease in activity of uroporphyrinogen decarboxylase (URO-D) and an increase in 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. Coplanar PCB congeners are more potent inhibitors of URO-D activity in cultured chicken embryo liver cells than are non-coplanar PCB congeners, 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 any increase in accumulation of uroporphyrins in chicken embryos. Thus, TCDD-induced lethality in chicken embryos is not associated with 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 via 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% of treated embryos vs. 29% of control embryos. 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.

In the chicken embryo heart, arachidonic acid metabolism is stimulated by TCDD, resulting in increased formation of prostaglandins. Dose-response relationships for the release of immunoreactive PGE₂, PGF₂α, and TXB₂ from chick embryonic heart are biphasic, with an apparent maximally effective dose of 100 pmol TCDD/egg. When the egg TCDD dose is increased further, release of these prostaglandins tends to decline toward levels in control hearts. Biphasic dose-response curves for cardiac PGE₂ release also were obtained with 3,3',4,4'-TCB and 3,3',4,4',5,5'-HCB. 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 lymphoid development of both of these immune system organs. Cultured thymus anlage from chick embryos are 100 times more
sensitive to the inhibitory effect of TCDD on lymphoid development than cultured thymus anlage from turkey and duck embryos.\(^{38}\) 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\(^{36}\) 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 rather than the immunotoxic effect of this coplanar PCB.

Within the same bird species, the signs of developmental toxicity elicited by TCDD and its approximate isomers 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, microphthalmia, beak deformities, and mortality; and TCDD, 3,3',4,4'-TCB and 3,3',4,4'-TCAOB inhibit lymphoid development.\(^{32,37-39}\) In pheasant embryos, an altogether different pattern of responses is seen. Nevertheless, TCDD-like congeners, TCDD, and 3,3',4,4'-TCB, injected into fertilized pheasant eggs, 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.\(^{31,51}\)

The lethal potency of TCDD and its approximate isomers 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.\(^{31,36,41,42,51,52}\) 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 in turkey embryos.\(^{30,31,36}\) In chicken embryos, an egg dose of 4 µg/kg 3,3',4,4'-TCB 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 1000 µg/kg egg had no effect on embryo mortality in domestic ducks, domestic geese, golden eye, herring gulls, and black-headed gulls.\(^{31,50}\) In contrast to the above-mentioned 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.\(^{53}\)

Graded doses of TCDD have been administered to fertilized eastern bluebird and ring-necked pheasant eggs for the purpose of determining the 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 1000 pg TCDD/g egg.\(^{42}\) For ring-necked pheasants, the LOAEL was 100 pg TCDD/g egg and the NOAEL was 10 pg TCDD/g egg. The LD\(_{50}\) for embryo mortality in the ring-necked pheasant is 1354 pg TCDD/g egg when the dose is injected into the egg albumin and 2182 pg TCDD/g egg when the dose is injected into the egg yolk.\(^{31}\) In contrast, for chickens, the LD\(_{50}\) for embryo mortality is 240 pg TCDD/g egg.\(^{30}\)

### 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 similar across species. The LD\(_{50}\) of TCDD in adult hamsters, 1157 to 5051 µg/kg, makes adult hamsters three orders of magnitude more resistant to TCDD-induced lethality than are adult guinea pigs.\(^{54-55}\) Yet, a maternal dose of 18 µg TCDD/kg can increase the incidence of prenatal mortality in the hamster embryo/fetus. Because this dose is only 12-fold larger than the 1.5 µg TCDD/kg dose 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.\(^{56-57}\) 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 1). Given a particular dosage regimen, the response is dose...
<table>
<thead>
<tr>
<th>Species/strain</th>
<th>Daily TCDD dose (µg/kg/day)</th>
<th>Cumulative TCDD dose (µg/kg)</th>
<th>Overt maternal toxicity</th>
<th>Percent prenatal mortality</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey/rhesus</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−</td>
<td>25</td>
<td></td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>−</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>+&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>+&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea pig/Hartley</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>−</td>
<td>−</td>
<td></td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>−</td>
<td>−</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit/New Zealand</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>−</td>
<td>7</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>1</td>
<td>−</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>2.5</td>
<td>+</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>5</td>
<td>+</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10</td>
<td>+</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Rat/Wistar</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>−</td>
<td>3</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>1.25</td>
<td>−</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>2.5</td>
<td>−</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>5</td>
<td>−</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10</td>
<td>±</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10</td>
<td>+</td>
<td>36&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20</td>
<td>+</td>
<td>53&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>40</td>
<td>+</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Rat/Sprague-Dawley</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>−</td>
<td>25</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>0.3</td>
<td>−</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>1.25</td>
<td>−</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>5</td>
<td>+</td>
<td>41&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20</td>
<td>+</td>
<td>96&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>80</td>
<td>+</td>
<td>100&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Hamster/Golden Syrian</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−</td>
<td>−</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>−</td>
<td>−</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>−</td>
<td>−</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>−</td>
<td>−</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>−</td>
<td>−</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Mouse/CD-1</td>
<td>0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
<td>−</td>
<td>7</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>250</td>
<td>−</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>500</td>
<td>−</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1000</td>
<td>−</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>2000</td>
<td>+</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>4000</td>
<td>+</td>
<td>97</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Decreased body weight gain or marked edema compared to vehicle-dosed controls. A (+) or (−) indicates the presence or absence of an effect.

<sup>b</sup> Percentage of absorptions plus late gestational deaths relative to all implantations. A (+) or (−) is given to indicate the presence of absence of an effect.

<sup>c</sup> TCDD administered in a single or divided doses between gestational days 20 and 40.

<sup>d</sup> 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.

<sup>e</sup> Single dose of TCDD administered on gestational day 14.

<sup>f</sup> TCDD administered daily on days 6–15 of gestation.

<sup>g</sup> Significant at P <0.05.

<sup>h</sup> Single dose of TCDD administered on gestational day 7 or 9.

<sup>i</sup> TCDD administered daily on days 7–16 of gestation.

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 1. This is 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 is 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 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 period of early development that results in the greatest incidence of prenatal toxicity. However, in nearly all species of adult laboratory mammals, 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 may result in prenatal mortality, whereas a single dose may 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 is applied to the data obtained by using different strains of mice. This then illustrates the importance of considering dosage regimen when evaluating published prenatal mortality data. In regard to prenatal mortality, a difference of one gestational day may be critically important. We show that the form of analysis using cumulative maternal dose gives the greatest possible degree of species variation. In turn, different species seem to actually be more similar with respect to susceptibility to prenatal mortality than would be superficially suggested by this type of analysis.

Using the cumulative dose data that are given in Table 1, there appears to be a 10- to 20-fold difference in the fetoletal potency of TCDD when the monkey/guinea pig is compared to the rabbit/rat/hamster. In the CD-1 mouse administered cumulative doses of TCDD on gestational days 7 to 16, not including day 6, it appears to require a daily dose of 200 \( \mu \text{g} \) TCDD/kg to significantly increase prenatal mortality. Given a TCDD half-life of approximately 5.5 days in the pregnant dam, the pregnant CD-1 mouse would be exposed to a maximal accumulated dose of approximately 1200 \( \mu \text{g} \) 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 ~1200-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 \( \mu \text{g} \) TCDD/kg. 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 a 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 \( \mu \text{g} \) TCDD/kg given on gestational day 6. This mouse strain therefore is about 20- to 30-fold 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.

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 14.60 Similarly, Neubert and Dillman59 found that the largest increase in prenatal mortality occurred when a single dose of TCDD was given on gestational day 6 compared to when the TCDD dose was administered on one of the 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 to the embryo/fetus of other species that would be indicated by using the cumulative maternal dose as the index of exposure.

Human pregnancies also are affected by critical periods during which the human embryo/fetus is susceptible to particular forms of chemical-induced developmental toxicity. In the first 2 weeks of human pregnancy, the predominant adverse developmental response is prenatal mortality, although other manifestations of developmental toxicity may occur during this period. The gestational period between weeks 2 through 8 is when the human embryo/fetus is most susceptible to the occurrence of structural malformations. Minor structural defects and postnatal functional alterations are the dominant adverse developmental effects that occur after 8 weeks of pregnancy.

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 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 does not seem 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 is the adult. The maternal dose of TCDD that causes 58% fetal mortality in hamsters is 64 to 280 times less than the LD_{50} of TCDD in adult hamsters.34,55,61 In Sprague-Dawley rats, the cumulative maternal dose of TCDD that causes 41% prenatal mortality is 5 to 10 times less than the approximate LD_{50} of TCDD in adult rats of the same strain.62,63 In rhesus monkeys, the cumulative maternal TCDD dose that causes 81% 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.54–66

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 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 metamyelocytes and bands, whereas increases in leukocyte number and bands also are found in maternal blood.56,57 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.67 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 be-
tween prenatal mortality and maternal toxicity. While only small numbers of monkeys have been studied to date, the results following dietary exposure to 25 ppt TCDD and 50 ppt TCDD 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 III.A.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. 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 3 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 is described in Section III.A.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 characterized. The integrity of the embryo-maternal vascular barrier, for example, has not been evaluated after TCDD exposure. It is possible that TCDD-induced developmental toxicity results from actions exerted on the mother, embryo/fetus, placenta, or any combination of these sites.

In most laboratory mammals, gestational exposure to TCDD produces a characteristic pattern of fetotoxic responses that consist of thymic hypoplasia, subcutaneous edema, decreased fetal growth, and prenatal mortality. In addition 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 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 sign 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. In the rat, TCDD prenatal toxicity is manifested by intestinal hemorrhage, subcutaneous edema, decreased fetal growth, and mortality. Structural abnormalities do occur in the rat but only at relatively large doses. In the hamster fetus, hydronephrosis and renal congestion are the most sensitive effects, followed by subcutaneous edema and mortality at fetolethal doses. In the rabbit, an increased incidence of extra ribs and prenatal mortality is found, whereas in the guinea pig and rhesus monkey, prenatal mortality is seen.

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, does not cause TCDD-like fetotoxic effects. On the other hand, hexachlorobenzeno-p-dioxin, which has intrinsic Ah receptor activity, produces fetotoxic responses in rats that are essentially identical to those of TCDD. Similarly, when administered to pregnant rhesus monkeys or CD-1 mice, PCB congeners that act via 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 essentially have no or very weak affinity for the Ah receptor, do not produce a TCDD-like pattern of prenatal toxicity in mice. 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 does TCDD.
<table>
<thead>
<tr>
<th>Species/strain</th>
<th>Daily dosea</th>
<th>Treatment daysb</th>
<th>Sacrifice dayb</th>
<th>Maternal effectsc</th>
<th>Embryo/fetal effectsd</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice/C57BL/6N</td>
<td>0, 1, or 3 μg/kg</td>
<td>10, 10–13</td>
<td>18</td>
<td>NR</td>
<td>Increase in cleft palate and hydropsphrosis</td>
<td>144</td>
</tr>
<tr>
<td>Mice/C57BL/6N</td>
<td>0, 12, 17, or 22 μg/kg</td>
<td>10</td>
<td>18</td>
<td>Increase in liver-to-body weight ratio</td>
<td>Increase in cleft palate and hydropsphrosis</td>
<td>102</td>
</tr>
<tr>
<td>Mice/C57BL/6N</td>
<td>0, 3, or 12 μg/kg</td>
<td>11, 10–13</td>
<td>18</td>
<td>Increase in liver-to-body weight ratio</td>
<td>Increase in cleft palate and hydropsphrosis</td>
<td>145</td>
</tr>
<tr>
<td>Mice/C57BL/6N</td>
<td>0 or 3 μg/kg</td>
<td>10–13</td>
<td>18</td>
<td>Increase in liver-to-body weight ratio</td>
<td>Increase in hydropsphrosis</td>
<td>277</td>
</tr>
<tr>
<td>Mice/C57BL/6N</td>
<td>0, 6, 9, 12, 15, or 18 μg/kg</td>
<td>10, 12</td>
<td>18</td>
<td>Increase in liver-to-body weight ratio and weight gain</td>
<td>Increase in cleft palate and hydropsphrosis</td>
<td>278</td>
</tr>
<tr>
<td>Mice/C57BL/6J</td>
<td>0 or 3 μg/kg (subcutaneous)</td>
<td>6–15</td>
<td>18</td>
<td>Increase in liver-to-body weight ratio</td>
<td>Increase in cleft palate and kidney anomaly</td>
<td>77</td>
</tr>
<tr>
<td>Mice/C57BL/6J</td>
<td>20 μg/kg</td>
<td>10</td>
<td>17</td>
<td>Increase in liver-to-body weight ratio</td>
<td>Increase in cleft palate, hydropsphrosis, and fetal body weight</td>
<td>273</td>
</tr>
<tr>
<td>Mice/C57BL/6J</td>
<td>0, 0.5, 1, 2, or 4 μg/kg</td>
<td>6–15</td>
<td>18</td>
<td>Increase in liver-to-body weight ratio</td>
<td>Increase in cleft palate, hydropsphrosis, and fetal body weight</td>
<td>149</td>
</tr>
<tr>
<td>Mice/NMR</td>
<td>0.3, 3, 4, 5, or 9 μg/kg</td>
<td>6–15</td>
<td>18</td>
<td>NR</td>
<td>Increase in cleft palate and fetal mortality; decrease in fetal body weight</td>
<td>59</td>
</tr>
<tr>
<td>Mice/CF-1</td>
<td>0, 0.001, 0.01, 0.1, or 1.3 μg/kg</td>
<td>6–15</td>
<td>NR</td>
<td>None</td>
<td>Increase in cleft palate and hydropsphrosis</td>
<td>276</td>
</tr>
<tr>
<td>Mice/DBA</td>
<td>0 or 3 μg/kg (subcutaneous)</td>
<td>6–15</td>
<td>18</td>
<td>Increase in liver-to-body weight ratio</td>
<td>Increase in cleft palate and kidney anomaly</td>
<td>77</td>
</tr>
<tr>
<td>Mice/DBA</td>
<td>0, 0.5, 2, 4, or 8 μg/kg</td>
<td>6–15</td>
<td>18</td>
<td>Increase in liver-to-body weight ratio; decrease in thymus-to-body weight ratio</td>
<td>Increase in cleft palate and hydropsphrosis</td>
<td>149</td>
</tr>
<tr>
<td>Mice/CD-1</td>
<td>0, 1, or 3 μg/kg (subcutaneous)</td>
<td>6–15</td>
<td>17</td>
<td>None</td>
<td>Increase in cleft palate and kidney anomaly</td>
<td>77</td>
</tr>
<tr>
<td>Mice/CD-1</td>
<td>0, 25, 50, 100, 200, or 400 μg/kg</td>
<td>6–15</td>
<td>17</td>
<td>Increase in liver-to-body weight ratio</td>
<td>Increase in cleft palate, hydropsphrosis, and fetal mortality</td>
<td>76</td>
</tr>
<tr>
<td>Rats/CD</td>
<td>0 or 0.5 μg/kg 2 μg/kg (subcutaneous)</td>
<td>6–15, 9–10 or 13–14</td>
<td>20</td>
<td>None at 0.5 μg/kg</td>
<td>NR at 2 μg/kg</td>
<td>Increase in kidney anomaly</td>
</tr>
<tr>
<td>Rats/Sprague-Dawley</td>
<td>0, 0.125, 0.5, or 2 μg/kg</td>
<td>0–2</td>
<td>20</td>
<td>Decrease in weight gain</td>
<td>Decrease in fetal body weight</td>
<td>274</td>
</tr>
<tr>
<td>Rats/Sprague-Dawley</td>
<td>0.03, 0.125, 0.5, 2, or 8 μg/kg</td>
<td>6–15</td>
<td>21</td>
<td>Decrease in weight gain; toxicity</td>
<td>Increase in fetal mortality, edema, and gastrointestinal hemorrhage</td>
<td>62</td>
</tr>
<tr>
<td>Rats/Wistar</td>
<td>0, 0.125, 0.25, 0.5, 1, 2, 4, 8, or 16 μg/kg</td>
<td>5–14</td>
<td>21</td>
<td>Toxicity</td>
<td>Increase in fetal mortality, edema, and gastrointestinal hemorrhage; decrease in fetal weight</td>
<td>78</td>
</tr>
<tr>
<td>Guinea pigs/Hartley</td>
<td>0, 0.15, or 1.5 μg/kg</td>
<td>14</td>
<td>58</td>
<td>Increase in mortality; toxicity</td>
<td>Increase in fetal mortality</td>
<td>56</td>
</tr>
<tr>
<td>Hamsters/Golden Syrian</td>
<td>0, 1.5, 3, 6, or 18 μg/kg</td>
<td>7, 9</td>
<td>15</td>
<td>Increase in liver-to-body weight ratio</td>
<td>Increased fetal mortality, hydropsphrosis, and renal congestion; decreased thymus size</td>
<td>56</td>
</tr>
<tr>
<td>Rabbits/New Zealand</td>
<td>0, 0.1, 0.25, 0.5, or 1 μg/kg</td>
<td>6–15</td>
<td>28</td>
<td>Decrease in weight gain; toxicity</td>
<td>Increase in fetal mortality and resorptions; extra ribs</td>
<td>79</td>
</tr>
</tbody>
</table>
### TABLE 2 (continued)
Developmental Toxicity Following Gestational Exposure to 2,3,7,8-TCDD

<table>
<thead>
<tr>
<th>Species/strain</th>
<th>Daily dose*</th>
<th>Treatment daysa</th>
<th>Sacrifice dayb</th>
<th>Maternal effectsc</th>
<th>Embryo/fetal effectsd</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus monkeys</td>
<td>0, 5, 25, 50, or 500 ppt 7 months before and during pregnancy</td>
<td>Chronic</td>
<td>—</td>
<td>Increase in mortality; toxicity</td>
<td>Increase in fetal mortality</td>
<td>71, 223</td>
</tr>
<tr>
<td>Monkeys/rahesus</td>
<td>0, 0.2, 1, 1, or 5 μg/kg</td>
<td>20–40</td>
<td>—</td>
<td>Increase in mortality; toxicity</td>
<td>Increase in fetal mortality</td>
<td>65</td>
</tr>
</tbody>
</table>

Note: NR = Not reported.

* Oral exposure unless otherwise noted.

* All days adjusted to reflect plug day — gestational day 0.

* Effects reported are only those that were statistically significant.

* Cumulative dose divided into nine oral doses administered between days 20 and 40 of gestation; two to four monkeys/dose.

* Three animals given single oral dose on either gestational days 25, 30, 35, or 40; 12 monkeys total.


### 5. Humans

In the Yusho and Yu-Cheng poisoning episodes, developmental toxicity was reported in babies born to affected mothers who had consumed rice oil contaminated with PCBs, CDFs, and PCQs. In these incidents, it is essentially impossible to determine the contribution of TCDD-like vs. nonTCDD-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. 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. Based on this evidence, Rogan 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, MO, TCDD incidents.

In laboratory mammals, the studies summarized previously in Table 1 indicate 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 or not there is any association between overt maternal toxicity and embryo/fetal toxicity both in 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: (1) fetal death, (2) structural malformations, (3) organ system dysfunction, and (4) growth retardation. In the Yusho and/or Yu-Cheng incidents, all of these outcomes were found. Increased prenatal mortality and low birth weight suggesting fetal growth retardation were observed in affected Yusho and Yu-Cheng women. A structural malformation, rocker bottom heel, was observed in Yusho infants. Organ dysfunctions involving the central nervous system (CNS), which were characterized by delays in attaining developmental milestones and neurobehavioral abnormalities, were reported in Yu-Cheng children exposed transplacentally.
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.\textsuperscript{70} Similarly, a hallmark sign of fetal/neonatal toxicity in the Yusho and Yu-Cheng episodes was an ectodermal dysplasia syndrome, which is characterized by hyperpigmentation of the skin and mucous membranes, hyperpigmentation and deformation of finger and toe nails, hypersecretion of the meibomian glands, conjunctivitis, gingival hyperplasia, presence of erupted teeth in newborn infants, and altered eruption of permanent teeth, missing permanent teeth, and abnormally shaped tooth roots.\textsuperscript{15,17,84,88,91,92,94–96} Accelerated tooth eruption has been observed in newborn mice exposed to TCDD by lactation,\textsuperscript{97} as well as in the human infants mentioned previously. In addition, other effects have been reported in the Yusho and Yu-Cheng exposed infants that resemble effects observed following TCDD exposure in adult monkeys. These include subcutaneous edema of the face and eyelids.\textsuperscript{84,89,92} Also, larger and wider fontanels, and abnormal lung auscultation were found in the human infants.\textsuperscript{84,89,92} The similarities between certain effects reported in human infants exposed during the Yusho and Yu-Cheng incidents, as well as adult monkeys and neonatal mice exposed to TCDD, enhance the probability that certain effects reported in the human infants were caused by the TCDD-like PCB and CDF congeners in the contaminated rice oil ingested by the mothers of these infants.

Chloracne is the most often cited effect of TCDD exposure involving the skin in adult humans. This effect has an animal correlate in the hairless mouse and can be studied by using a mouse teratoma cell line in tissue culture.\textsuperscript{7} Nevertheless, it has rarely been explicitly recognized in the TCDD literature that the nervous system, like the skin, is derived from embryonic ectoderm.\textsuperscript{99} As is described in Section II.C.2, neurobehavioral effects occur following transplacental and neonatal exposure to TCDD-like congeners in mice, as well as transplacental exposure to TCDD in monkeys. In addition, some of the Yu-Cheng children that were exposed transplacentally to PCBs, PCDFs, and PCQs have affected a clinical impression of developmental delay or psychomotor delay including impairment of intellectual development.\textsuperscript{92,93} Because there is a clustering of effects due to TCDD-induced toxicity in organs derived from ectoderm, it is reasonable to speculate that direct effects of TCDD-like congeners on the CNS 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\textsuperscript{100} and accelerated tooth eruption.\textsuperscript{97} Decreased autophosphorylation of the EGF receptor in human placentas is associated with decreased birth weight in infants born to exposed mothers 4 years after the initial Yu-Cheng exposure incident.\textsuperscript{101} 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.

### B. Structural Malformations

Developmental effects consisting of cleft palate, hydernephrosis, and thymic hypoplasia are produced in mice following in utero exposure to halogenated dibenzo-p-dioxin, dibenzofuran, and biphenyl and naphthalene congeners, which bind stereospecifically to the Ah receptor.\textsuperscript{102–105} Of these effects in the mouse, cleft palate is less responsive than hydrenephrosis inasmuch as the latter is induced in the absence of cleft palate.\textsuperscript{60} Both responses can be induced at TCDD doses that are not otherwise overtly toxic.\textsuperscript{75} The potency of TCDD for producing teratogenesis in the mouse is clearly evident when one considers that only 0.0003% 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.\textsuperscript{106}
Susceptibility to the developmental toxicity of TCDD in mice depends on two factors: the genotype of the fetus and the stage of development at the time of exposure. Because 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, the Ah receptor is thought to mediate the developmental effects of TCDD. 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 because all strains, including those with Ah receptors of relatively low affinity, respond when exposed to sufficiently large doses during the critical period of organogenesis. 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.

In inbred strains of mice, the developmental response, characterized by altered cellular proliferation, metaplasia, and modified terminal differentiation of epithelial tissues, is extremely organ specific, occurring only in the palate, kidney, and thymus. Pharmacokinetic differences are not responsible for this high degree of tissue specificity, and Ah receptors are not found exclusively in the affected organs. 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 various developmental effects, the time at which exposure occurs is important and defines the critical period during which the toxicant must be present in order to produce the effect. This critical period differs among organs and tissues within organs.

Differences exist between mammalian species with respect to susceptibility to the developmental effects of TCDD. Although genetic differences between species or strains may affect absorption, biotransformation, and/or elimination of TCDD by the maternal system and its transport across the placenta, such species differences do not account for the lack of cleft palate formation in species other than mice. Rather, the species differences in susceptibility to cleft palate formation appear to be 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. The key difference is that, relative to the concentration of TCDD that affects murine palatal shelves in culture, much higher concentrations of TCDD are required to elicit the same effects in cultured palatal shelves from other species (Table 3). As a result, it appears that differences in the responsiveness of palatal tissue to the effects of TCDD explain the absence of cleft palate in nonmurine species except at maternal doses that are fetotoxic and maternally toxic.

In mice and hamsters, hydrocephalus can be elicited at TCDD doses that are neither fetotoxic nor maternally toxic, whereas thymic hypoplasia is a fetal response to TCDD observed in virtually all laboratory mammalian species tested. Studies in humans have not clearly identified an association between TCDD exposure and structural malformations.

1. Cleft Palate

a. 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. 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
TABLE 3
TCDD Responsiveness of Palatal Shelves from the Mouse, Rat, and Human in Organ Culture

<table>
<thead>
<tr>
<th>Species</th>
<th>LOEL</th>
<th>EC&lt;sub&gt;100&lt;/sub&gt;</th>
<th>Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>1 x 10&lt;sup&gt;-13&lt;/sup&gt;</td>
<td>5 x 10&lt;sup&gt;-11&lt;/sup&gt;</td>
<td>1 x 10&lt;sup&gt;-10&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rat&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1 x 10&lt;sup&gt;-10&lt;/sup&gt;</td>
<td>1 x 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>1 x 10&lt;sup&gt;-7&lt;/sup&gt;</td>
</tr>
<tr>
<td>Human&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5 x 10&lt;sup&gt;-11&lt;/sup&gt;</td>
<td>1 x 10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>1 x 10&lt;sup&gt;-7&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> At the highest concentration tested, 60% of the palatal shelves failed to undergo the transformation process.

<sup>b</sup> One of four shelves responded by failing to undergo the transformation process at 5 x 10<sup>-11</sup> M.


cells that are left within the seam. The midline seam thus formed consists of two healthy-appearing layers of basal cells (one on each side of the seam), whereas the outer periderm cells have already been shed. As the palatal shelf continues to grow, the bilayer seam, which itself grows at a slower rate, turns into a single layer of cells, and then 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 above-described process makes the medial edge epithelium an ectoderm that retains the ability to transform into mesenchyme. 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.<sup>120,121</sup>

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.<sup>122</sup> TCDD and other Ah receptor agonists act by allowing the shelves to grow and make contact, but the subsequent process involving 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.<sup>60</sup>

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 <i>in vitro</i>, sloughing periderm cells are trapped within the seam.<sup>120</sup> Thus, due to the presence of these trapped dead cells, the fusion process was originally described as a process of programmed cell death.<sup>118,119,123</sup> However, the newer idea, which presumes transformation of the basal epithelial cells into mesenchyme rather than their death, is believed to be valid under explant conditions <i>in vitro</i>, as well as <i>in vivo</i>.<sup>120</sup> When exposed to TCDD as explants <i>in vitro</i>, the palatal shelves of the mouse, rat, and human all respond to TCDD in a similar way by not completing the fusion process.<sup>106,112,113,124</sup> There is death of the outer peridermal cells, after which, the epithelial-to-mesenchyme transformation of the basal epithelial cells does not occur. Instead, there is a differentiation of these basal cells into a stratified squamous epithelium such that they eventually
resemble the squamous keratinizing oral cells within the tissue.\textsuperscript{125}

Table 3 shows the lowest TCDD concentration that prevents the epithelial-to-mesenchyme transformation process in isolated palatal shelves (LOEL), the TCDD concentration that produces a 100% maximal response (EC\textsubscript{100}), and the 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 prevent the epithelial-to-mesenchyme transformation process. The relative insensitivity of rat palatal shelves may explain the lack of cleft palates 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-\(\alpha\), TGF-\(\beta1\), and TGF-\(\beta2\) 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-\(\alpha\). Also, decreased levels of immunohistochemically detectable TGF-\(\beta1\) could contribute to the continued proliferation and altered differentiation of medial cells.\textsuperscript{126} It is important to note that EGF and TGF-\(\alpha\) 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 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.\textsuperscript{100} 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,\textsuperscript{120} in that the medial edge epithelium within the developing palate is essentially an ectoderm that retains the ability to transform into mesenchymal cells.

\textbf{b. Evidence for an Ah Receptor Mechanism}

\textbf{i. Genetic}

When wild-type C57BL/6 (Ah\textsuperscript{a}Ah\textsuperscript{b}) mice are crossed with DBA/2 (Ah\textsuperscript{a}Ah\textsuperscript{d}) mice that contain a mutation at the Ah locus, all of the heterozygous B6D2F1 progeny (Ah\textsuperscript{a}Ah\textsuperscript{d}) resemble the wild-type parent in that arylhydrocarbon hydroxylase (AHH) activity is inducible by TCDD and other halogenated aromatic hydrocarbons.\textsuperscript{127} 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\textsubscript{2} progeny. Thus, the trait of AHH inducibility is expressed in progeny that contain the Ah\textsuperscript{a}Ah\textsuperscript{b} and Ah\textsuperscript{a}Ah\textsuperscript{d} genotypes, but is not expressed in the Ah\textsuperscript{a}Ah\textsuperscript{d} progeny from these crosses. Certain other effects of TCDD, such as its binding affinity for the hepatic Ah receptor,\textsuperscript{128} thymic atrophy,\textsuperscript{107} hepatic porphyria,\textsuperscript{129} and immunosuppressive effects,\textsuperscript{130,131} have been shown in similar genetic crosses and test crosses to segregate with the Ah locus, which permits AHH induction. Thus, for these effects of TCDD, genetic evidence demonstrates an involvement of the Ah locus.\textsuperscript{3}

Nebert’s group was the first to relate developmental toxicity to the Ah locus in mice.\textsuperscript{132,133} Subsequently, Poland and Glover\textsuperscript{107} administered a single 30-\(\mu\)g TCDD/kg dose to pregnant mice on gestational day 10. It was found that there was a 54% incidence of cleft palate in homozygous C57BL/6 (Ah\textsuperscript{a}Ah\textsuperscript{b}) fetuses, a 13% incidence in heterozygous B6D2F1 (C57BL/6 and DBA/2 hybrid, Ah\textsuperscript{a}Ah\textsuperscript{d}) fetuses, and only a 2% incidence in homozygous DBA/2 (Ah\textsuperscript{a}Ah\textsuperscript{d}) fetuses. This pattern of inheritance, in which the incidence of developmental toxicity in the heterozygous F\textsubscript{1} 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, even if dominance is incomplete in the case of developmental toxicity. However, the pattern of inheritance for developmental toxicity described when Poland and Glover crossed C57BL/6 and DBA/2 mice is not proof positive that the Ah locus is the only 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 earlier in this section 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 is now described. It is intended that this information contain a considerable degree of detail. This is done so that the reader can independently determine whether or not the standard of proof has been satisfied by the evidence available.

In order to strengthen their conclusion based on the results of simple crosses between C57BL/6 and DBA/2 mice, Poland and Glover 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 AhAH and AhAH 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 examined the incidence of cleft palate in ten inbred strains of mice exposed to TCDD: five strains with high-affinity Ah receptors and five strains with low-affinity receptors. In the five latter strains, there was only a 0 to 3% incidence of cleft palate formation, whereas four of the five strains with high-affinity Ah receptors developed a 50% incidence. The one strain with high-affinity Ah receptors that did not follow the pattern, the CBA strain, also is resistant to cleft palate formation induced by glucocorticoids and may therefore be generally resistant to chemically induced cleft palate formation. 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. 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%, 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. 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,\textsuperscript{107} Hassoun et al.\textsuperscript{108} were unable to determine whether or not 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\textsubscript{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\textsubscript{42} and F\textsubscript{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%. After similar matings with eight different BXD strains with low-affinity Ah receptors, the incidence of TCDF-induced cleft palate formation is <2%. These results of Hassoun et al.\textsuperscript{108} corroborate those of Poland and Glover\textsuperscript{107} and provide the best evidence currently available that cleft palate formation segregates with the Ah locus. Accordingly, 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\textsuperscript{a}Ah\textsuperscript{b}, C57BL/6 fetuses, but are not detectable in similar tissue from Ah\textsuperscript{b}Ah\textsuperscript{b}, AKR/J fetuses.\textsuperscript{136} 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 to 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 numbers 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 \textsuperscript{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.\textsuperscript{137}

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.\textsuperscript{138} After administration of 30 \textmu g TCDD/kg or 8 mg TCAOB/kg to pregnant dams on gestational day 12, 75 to 100% of all NMRI fetuses developed cleft palates. This was 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% of their NMRI littermates 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 the direct effect of TCDD on the palatal shelf itself and is not secondary to maternal toxicity.

ii. Structure Activity

Because the genetic evidence in mice indicates that the Ah receptor mediates TCDD-induced cleft palate formation and hydronephrosis (see Sections II.B.1.b.i and II.B.2.b.i), 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 4 shows the relative potencies for cleft palate induction and hydronephrosis in C57BL/6 mice for a number of TCDD-like congeners. Because TCDD is the most potent, it is assigned a value of 1.000. When examined using probit analysis, the dose-response curve of each congener, compared to all of the others, did not deviate from parallelism. Therefore, the relative
TABLE 4
Relative Teratogenic Potency of Halogenated
Aromatic Hydrocarbon Congeners in C57BL/6
Mice

<table>
<thead>
<tr>
<th>Congener</th>
<th>Relative potency (ED_{50} TCDD/ED_{50} Congener)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cleft palate</td>
</tr>
<tr>
<td>2,3,7,8-TCDD</td>
<td>1.000</td>
</tr>
<tr>
<td>2,3,7,8-TBDD</td>
<td>0.235</td>
</tr>
<tr>
<td>2,3,7,8-TBDF</td>
<td>0.100</td>
</tr>
<tr>
<td>2,3,4,7,8-PeCDF</td>
<td>0.095</td>
</tr>
<tr>
<td>2,3,7,8-TCDF</td>
<td>0.049</td>
</tr>
<tr>
<td>1,2,3,7,8-PeCDF</td>
<td>0.026</td>
</tr>
<tr>
<td>1,2,3,4,7,8-HxCDF</td>
<td>0.010</td>
</tr>
<tr>
<td>2,3,4,7,8-NeCDF</td>
<td>0.005</td>
</tr>
<tr>
<td>1,2,3,7,8-NeBDF</td>
<td>0.004</td>
</tr>
<tr>
<td>2,3,4,5,3',4'-HxCB</td>
<td>0.0000287</td>
</tr>
</tbody>
</table>

From References 102–104 and 109.

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 (Table 4), with the notable exception that the apparent binding affinities for the brominated dibenzo-furans 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'-TCOAB, 3,3',4,4'-tetrachlorobiphenyl, 3,3',4,4',5,5'-hexachlorobiphenyl, and a mixture that contained 1,2,3,4,6,7- and 2,3,4,5,6,7-hexabromonaphthalenes.

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 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. In addition, it is consistent with the structure-activity relationships that monoortho-chlorine-substituted 2,3,4,5,3',4'-HCB is a weak teratogen. Its potency relative to that of TCDD varies from 3 \times 10^{-5} to 9 \times 10^{-5} for cleft palate formation, AHH induction, and hydronephrosis (Table 4).

A result that would not be expected according to the structure-activity relationships for binding to the Ah receptor is that diortho-chlorine-substituted 2,2',3,3',4,4'-hexachlorobiphenyl causes cleft palate formation and hydronephrosis in mice. 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 7-ethoxyresorufin-O-deethylase (EROD) activity. 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, that it can inhibit TCDD-induced cleft palate formation and immunotoxicity in C57BL/6 mice. These results suggest that PCB congeners do not have to be in a strictly planar configuration to cause teratogenesis.

c. Species Differences

Cleft palate is induced in rats only at maternally toxic TCDD doses that are associated with a high incidence of fetal lethality. Schuetz et al. reported an increased incidence of cleft palate after maternal administration of 100 \mu g hexachlorodibenzo-p-dioxin/kg/day on days 6 to 15 of gestation to Sprague-Dawley rats. Couture et al. also observed an increased incidence of cleft palate formation after a single dose of 300 \mu g/kg of 2,3,4,7,8-pentachlorodibenzo-furan given to Fisher 344 rats. Similarly, cleft palate can be produced in fetal hamsters following maternally toxic and fetotoxic doses of TCDD.

In monkeys, bifi uvula and bony defects in the hard palate 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. 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.
2. Hydronephrosis

a. 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.\(^{77,102,144-147}\) Hyperplasia of the ureteric luminal 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.\(^{146}\)

When dissected on gestational day 12 from control embryos, isolated ureters exposed to \(1 \times 10^{-10} M\) TCDD \textit{in vitro} display evidence of epithelial cell hyperplasia.\(^{148}\) This is significant in that it shows that the hydronephrosis response is due to the 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.\(^{148}\) 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 EGF receptor numbers do not decline. Therefore, in TCDD-treated mice, there is a correlation between excessive proliferation of ureteric epithelial cells and increased expression of EGF receptors.

Other effects of TCDD on the developing kidney involve changes in extracellular matrix components and basal lamina.\(^{147}\) 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 also is 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, i.e., 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 appear to involve ectoderm-derived organs. It is not intended to imply that all TCDD-induced developmental toxicity involves organs derived from ectoderm.

b. Evidence for an Ah Receptor Mechanism

i. 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, Courtney and Moore\(^ {77}\) reported a 62% incidence of hydronephrosis in C57BL/6 mice exposed to a maternal TCDD dose of 3 g/kg/day on days 6 to 15 of gestation, whereas the incidence in similarly exposed DBA/2 mice was only 26%. More recently, Silkworth et al.\(^ {149}\) 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 g/kg/day, the incidence of hydronephrosis in C57BL/6 mice increases from 31 to 92%; in DBA/2 mice, the incidence varies from 5 to 37% over the same dose range. In DBA/2 mice, the incidence of hydronephrosis increases to 60% when the largest dose of TCDD administered is doubled to 8 g/kg/day (but does not reach the 92% level seen in C57BL/6 mice at 4 g/kg TCDD/kg). Thus, the incidence of hydronephrosis is higher in the mouse strain that produces high-affinity Ah receptors...
(C57BL/6) compared to the strain (DBA/2) that produces Ah receptors having lower ligand-binding affinity. The largest dose of TCDD used in these experiments resulted in hydropnephrosis of the fetus without affecting the mean body weight or body weight gain of the dam. In the BXD strains, the incidence of 2,3,7,8′-TCDF-induced hydropnephrosis is 34 to 48% in eight strains with high-affinity Ah receptors and 3 to 4% 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 hydropnephrosis and the wild-type Ah<sup>+</sup> allele. Accordingly, the Ah locus and the Ah receptor are involved in the hydropnephrosis that is induced by TCDD-like congeners.

**C. Postnatal Effects**

**1. Male Reproductive System of Rats**

Because TCDD can decrease plasma androgen concentrations and be transferred from mother to young in utero and during lactation, it is expected to have a great impact on the male reproductive system during early development. Testosterone and/or its metabolite DHT are essential prenatally and/or early postnaturally for imprinting and development of accessory sex organs and for initiation of spermatogenesis. In addition, aromatization of testosterone to 17β-estradiol within the CNS is required perinatally for the imprinting of typical adult male patterns of reproductive behavior and LH secretion. Thus, normal development of male reproductive organs and imprinting of typical adult sexual behavior patterns require sufficient testosterone be secreted by the fetal and neonatal testis at critical times in early development before and shortly after birth. If perinatal imprinting fails to occur in the accessory sex organs of a neonatal male rat, the results may be that these organs will not develop a normal trophic response to androgenic stimulation and will not function normally as the animal becomes sexually mature.

**a. Perinatal Androgen Deficiency**

To determine if in utero and lactational exposure to TCDD produces a perinatal androgenic deficiency, Mably et al. 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 h after birth in control males, whereas in TCDD-exposed males, the peak did not occur until 4 h after birth and was only half as large. Thus, in male rats, perinatal exposure to TCDD can produce both prenatal and early postnatal androgenic deficiencies.
b. Overt Toxicity Assessment

To determine how the male reproductive system is affected by in utero and lactational TCDD exposure. Mably et al. 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. The pups were weaned 21 days after birth, and the consequences of this single, maternal TCDD exposure for the male offspring were characterized at various stages of postnatal sexual development.

Mably et al. found that TCDD treatment had no effect on daily feed intake during pregnancy and the first 10 days after delivery, nor did it have any 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% 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% decrease in live birth index (highest dose only), the initial 10 to 15% decreases in body weight (two highest doses), and the initial 10 to 20% 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. No male or female offspring with gross external malformations were found.

c. Androgenic Status

Androgenic status of the male offspring, which includes such parameters as plasma androgen concentrations and androgen-dependent structures and functions, was reduced by a single maternal TCDD dose as low as 0.16 µg/kg. Anogenital distance, which is dependent both on circulating androgen concentrations and androgenic responsiveness, was reduced in 1- and 4-day-old male pups, even when slight decreases in body length were considered. Testis descent, an androgen-mediated development event that normally occurs in rats between 20 and 25 days of age, was delayed ≤1.7 days.

For accessory sex organs of an adult male rat to grow normally and respond fully to androgens, there is a critical period that starts before birth and lasts until sexual maturity during which adequate concentrations of androgens are necessary. To determine if perinatal TCDD exposure affects postnatal growth of the accessory sex organs, one rat from each litter was sacrificed at 32, 49, 63, and 120 days of age, corresponding to juvenile, pubertal, post-pubertal, and mature stages of sexual development, respectively. At each developmental stage, dose-related decreases in seminal vesicle and ventral prostate weights were found. These decreases could not be explained by decreases in body weight.

There were trends (although not statistically significant) for plasma testosterone and DHT concentrations to be decreased at these times, whereas plasma LH concentrations were generally unaffected. An exception was a 95% 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 bodyweight of male pups at this dose, 0.16 µg TCDD/kg caused a consistent pattern of effects that indicated 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.
Collectively, these results demonstrate that in utero and lactational TCDD exposure decreases androgenic status of male rats from the fetal stage into adulthood. Table 5 summarizes these effects.154,163

d. Spermatogenesis

Mably et al.154,165 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 approximately 45%. The number of sperm per cauda epididymis was decreased by 75 and 65% 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% at puberty, day 49, but the decrease was only 29% 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 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, although not completely, thus suggesting that perinatal TCDD exposure delays sexual maturation. These results are summarized in Table 6.154,165

Severe pre- and/or postweaning undernutrition can affect the reproductive system of adult male rodents, including decreased spermatogenesis.173-176 At the two highest maternal TCDD doses, the feed consumption and body weight of male offspring were decreased; even so, the weight decreases did not exceed 21% of the control values.163 However, reductions 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,

**TABLE 5**

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

<table>
<thead>
<tr>
<th>Index</th>
<th>Lowest effective maternal dose (μg TCDD/kg)*</th>
<th>Maximum effect**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anogenital distance</td>
<td>0.16 (days 1 and 4)</td>
<td>21% decrease (day 1)</td>
</tr>
<tr>
<td>Time to testis descent</td>
<td>0.16</td>
<td>1.7-day delay</td>
</tr>
<tr>
<td>Plasma testosterone concentration</td>
<td>NS</td>
<td>69% decrease (day 32)</td>
</tr>
<tr>
<td>Plasma 5α-dihydrotestosterone concentration</td>
<td>NS</td>
<td>59% decrease (day 49)</td>
</tr>
<tr>
<td>Plasma LH concentration</td>
<td>1.0 (day 32)</td>
<td>95% decrease (day 32)</td>
</tr>
<tr>
<td>Absolute seminal vesicle weight</td>
<td>0.16 (days 32 and 63)</td>
<td>56% decrease (day 49)</td>
</tr>
<tr>
<td>Relative seminal vesicle weight</td>
<td>0.16 (day 63)</td>
<td>50% decrease (day 49)</td>
</tr>
<tr>
<td>Absolute ventral prostate weight</td>
<td>0.064 (day 32)</td>
<td>60% decrease (day 32)</td>
</tr>
<tr>
<td>Relative ventral prostate weight</td>
<td>0.16 (days 32 and 63)</td>
<td>53% decrease (day 32)</td>
</tr>
</tbody>
</table>

Note: NS = not statistically significant.

* 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.

** The magnitude of the greatest change seen in response to maternal dosing with 1.0 μg/kg TCDD and the day at which this effect was seen are shown.

* Weight of organ divided by body weight of rat.

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

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

Note: NS = not statistically significant.

* 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.

* The magnitude of the greatest change seen in response to maternal dosing with 1.0 µg/kg TCDD and the day on which this effect was seen are shown.


Undernutrition cannot account for these reproductive system effects, including the decreases in spermatogenesis observed at the lower maternal TCDD doses. 163, 165

Since FSH and testosterone are essential for quantitatively normal spermatogenesis, an alternative explanation for the decreases in daily sperm production is a decrease in FSH and/or testosterone levels. In rats, the length of one spermatogenic cycle is 58 days, 177-179 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, was not due to decreases in plasma FSH levels when the animals were 49 to 120 days of age. 165

Plasma testosterone concentrations in the same rats were reduced ≤69% by perinatal TCDD exposure, yet intratesticular testosterone concentrations must be reduced by at least 80% in rats before spermatogenesis is impaired. 180 Based on the magnitude of the reductions in plasma androgen concentrations, it was concluded that corresponding reductions in testicular testosterone production in perinatal TCDD-exposed offspring would probably not be severe enough to impair spermatogenesis. 163, 165

In normal rats, daily sperm production does not reach a maximum until 100 to 125 days of age, 181 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, 165 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 that decreased the daily sperm production in male rat offspring that were 300 days of age. 182 Be-
cause 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, 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 lepotene 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. Because Sertoli cells provide spermatogenic cells with functional and structural support and the upper limit of daily sperm production in adult rats is directly dependent on the number of Sertoli cells per testis, three possible mechanisms for the decrease in daily sperm production may be involved: TCDD could (1) increase the degeneration of cells intermediate in development between lepotene spermatocytes and terminal stage spermatids (the cell type used to calculate daily sperm production); (2) decrease post-lepotene spermatocyte cell division (meiosis); and/or (3) decrease the number of Sertoli cells per testis. 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.

e. Epididymis

The epididymis has two functions: in proximal regions, spermatozoa mature gaining the capacity for motility and fertility; in distal regions, mature sperm are stored before ejaculation. Mably et al. 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 post-pubertal rats 63 and 120 days of age 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 cannot be explained by effects on sperm production. Because epididymal growth is androgen dependent, a TCDD-induced androgen deficiency and/or decrease in androgen responsiveness of the epididymis could account for decreased size of the organ.

f. 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 days of age. The fertility index of the males is defined as the number of males impregnating females divided by the number of males mated. The two highest maternal TCDD doses decreased the fertility index of the male offspring by 11 and 22%, respectively. However, these decreases were not statistically significant, and at lower doses, the fertility index was not reduced. The gestation index, defined as the percentage of control dams mated with TCDD-exposed males that delivered at least one live offspring, also was 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 the survival and growth of their offspring. These results are summarized in Table 6.

Because rats produce and ejaculate 10 times more sperm than are necessary for normal fertility and litter size, 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, reproduc-
tive efficiency in human males is very low; the number of sperm per ejaculate is close to that required for fertility. Thus, measures of fertility using rats are not appropriate for low-dose extrapolation in humans. A percent reduction in daily sperm production in humans, similar in magnitude to that observed in rats, may reduce fertility in men.

**g. 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. 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 when castrated and primed with ovarian steroids. 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. The mechanism by which androgens cause sexual differentiation of the CNS is not completely understood. In the rat, it appears that 17β-estradiol, formed by the aromatization of testosterone within the CNS, is one of the principal active steroids responsible for mediating sexual differentiation; however, androgens also are involved.

**i. Demasculinization of Sexual Behavior**

Mably et al. 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 (Table 7). The number of mounts and intromissions (mounts with vaginal penetration) before ejaculation were increased by a maternal TCDD dose of 1.0 μg/kg. The same males exhibited 12- and 11-fold increases in mount and intromission latencies, respectively, and a 2-fold 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 <43% 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% 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.

Since 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, 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. Postpubertal plasma testosterone and DHT concentrations in littersmates of the rats evaluated for masculine sexual behavior were as low as 56 and 62%, respectively, of control. However, plasma testosterone concentrations that were only 33% of control were still sufficient to masculinize the sexual behavior of adult male rats. 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. 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. Thus, although some effects of
TABLE 7
Effects of In Utero and Lactational TCDD Exposure on Indices of Sexual Behavior and Regulation of LH Secretion in Adulthood

<table>
<thead>
<tr>
<th>Index</th>
<th>Lowest effective maternal dose (µg/kg TCDD)</th>
<th>Maximum effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masculine sexual behavior c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mount latency</td>
<td>0.16</td>
<td>1200% increase</td>
</tr>
<tr>
<td>Intromission latency</td>
<td>0.064</td>
<td>1100% increase</td>
</tr>
<tr>
<td>Ejaculatory latency</td>
<td>0.16</td>
<td>97% increase</td>
</tr>
<tr>
<td>Number of mounts</td>
<td>0.064</td>
<td>130% increase</td>
</tr>
<tr>
<td>Number of intromissions</td>
<td>1.0</td>
<td>38% increase</td>
</tr>
<tr>
<td>Copulatory rate (mounts plus intromissions/minute)</td>
<td>0.16</td>
<td>43% decrease</td>
</tr>
<tr>
<td>Postejaculatory interval</td>
<td>0.40</td>
<td>35% increase</td>
</tr>
<tr>
<td>Feminine sexual behavior c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lordosis quotient</td>
<td>0.16</td>
<td>300% increase</td>
</tr>
<tr>
<td>Lordosis intensity score</td>
<td>0.40</td>
<td>50% increase</td>
</tr>
<tr>
<td>Regulation of LH secretion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH surge</td>
<td>0.40</td>
<td>460% increase</td>
</tr>
</tbody>
</table>

* 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.
* The magnitude of the greatest change seen in response to maternal dosing with 1.0 µg/kg TCDD is shown (average of three trials for masculine behavior and two for feminine).
* Measured when the rats were ~60, 75, and 115 days of age.
* Feminine sexual behavior was measured following castration, estrogen priming, and progesterone administration. The rats were 170–184 days old.
* Number of times lordosis was displayed in response to a mount divided by the number of times each rat was mounted times 100.
* Because 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.


TCDD, such as decreased copulatory rate and prolonged latency until ejaculation, could be due to reduced sensitivity of the penis to sexual stimulation, the 12-fold increase in mount latency and increase in postejaculatory interval could not be explained by this mechanism.

ii. Feminization of Sexual Behavior

Mabley et al. 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 17β-estradiol benzoate, followed 42 h later by progesterone. At 4 to 6 h after the progesterone injection on 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% (control) to 54% by the highest maternal TCDD dose, 1.0 µg/kg (Table 7). Lordosis intensity scored after Hardy and DeBold as a (1) for light lordosis, (2) for moderate lordosis, and (3) for a full spinal dorsoflexion was increased in male rats by perinatal TCDD exposure.
The 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, the increased frequency of lordotic behavior was seen at a maternal TCDD dose of 0.16 μg/kg that 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.

iii. 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. 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 feminized. Table 7 summarizes sexual behavior and LH secretion results.

iv. Comparison to Other Ah Receptor-Mediated Responses

The induction of hepatic cytochrome P-450IA1 and its associated EROD activity is an extremely sensitive Ah receptor-mediated response 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. These results suggest that TCDD affects sexual behavior, gonadotropic 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 is such that a continuing presence of TCDD is not required to produce these effects in adulthood.

v. Possible Mechanisms and Significance

The most plausible explanation for the demasculinization and feminization of sexual behavior and feminization of LH secretion is that perinatal exposure to TCDD impairs sexual differentiation of the CNS. Undernutrition, altered locomotor activity, reduced sensitivity of the penis to sexual stimulation, and modest reductions in adult plasma androgen concentrations of the male offspring cannot account for these effects. On the other hand, exposure of the developing brain to testosterone, conversion of testosterone into 17β-estradiol within the brain, and events initiated by the binding of 17β-estradiol to its receptor are all critical for sexual differentiation of the CNS. If TCDD interferes with any of these processes during late gestation and/or early neonatal life, it could irreversibly desensitize and feminize sexual behavior and feminize the regulation of LH secretion in male rats in adulthood.

Treatment of dams on day 15 of gestation with 1.0 μg TCDD/kg significantly decreases plasma testosterone concentrations in male rat fetuses on days 18 and 20 of gestation and in male rat pups 2 h postpartum. Thus, the ability of maternal TCDD exposure to reduce prenatal and early postnatal plasma testosterone concentrations may account, in part, for the impaired sexual differentiation of male rats exposed perinatally to TCDD. Other mechanisms that may potentially contribute to the TCDD-induced impairment in CNS sexual differentiation are a decrease in the formation of 17β-estradiol from testosterone within the CNS, which is independent of the decrease in plasma testosterone concentrations, and/or a reduction in responsiveness of the CNS to estrogen during the critical period of
sexual differentiation. The latter mechanism is consistent with the Ah receptor-mediated antiestrogen action of TCDD, which is described in Section III.A.3 for rat and mouse uterus and for estrogen-responsive MCF-7 and Hepa 1c1c7 cells.

In utero and/or lactational exposure to TCDD may cause similar effects in other animal species, including nonhuman primates, in which sexual differentiation is under androgenic control. Although social factors may account for much of the variation in the sexually dimorphic behavior in humans, there is evidence that prenatal androgenization influences both the sexual differentiation of such behavior and the brain hypothalamic structure.

2. Neurobehavior

Because differentiated tissues derived from ectoderm, i.e., skin, conjunctiva, nails, and teeth, are sites of action of halogenated aromatic hydrocarbons in transplacently 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 is the fact that sexual differentiation of the CNS of adult male rats is irreversibly altered in a dose-related fashion by perinatal exposure to TCDD. As is shown later, in mice transplacently exposed to 3,3',4,4'-TCB, monkeys perinatally exposed to TCDD, and children transplacently exposed to a mixture of PCBs, CDFs, and PCQs in the Yu-Cheng incident, the CNS is affected by TCDD and other halogenated aromatic hydrocarbons. Thus, functional CNS alterations, which may or may not be irreversible, are observed following perinatal exposure to these chemicals.

Ah receptors have been identified in rat brain but may be associated with glial cells rather than neurons. Following administration of 14C-TCDD in the rat, the highest concentrations TCDD-derived radioactivity are found in the hypothalamus and pituitary. Much lower concentrations are found in the cerebral cortex and cerebellum. 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. Ah receptors appear to be absent in the human frontal cortex.

a. 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. 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. The brain pathology in adult mice exhibiting this syndrome consisted, in part, of alterations in synapses of the nucleus accumbens. 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. 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 et al. 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, 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 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. Cholinergic as well as dopaminergic systems in the brain are involved.

Of all the developmental and reproductive endpoints reported in this section for laboratory animals, the only ones that have not yet been demonstrated to occur following perinatal exposure to TCDD are the above-mentioned neurotoxic effects in mice. These have only been studied following perinatal exposure to 3,3',4,4'-TCB. In addition, there is no evidence yet to show that (1) 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) 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 to 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 may produce CNS effects by being converted to a hydroxylated metabolite that is neurotoxic. While there is no evidence for or against this hypothesis, there also is 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.

b. Monkeys

Schantz and Bowman and Bowman et al. 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. 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. However, the perinatal TCDD exposure did produce a specific, replicable deficit in cognitive function. 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. 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.

c. 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. 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%) Yu-Cheng children compared with 3 (3%) control children, and of a speech problem in 8 (7%) Yu-Cheng children vs. 3 (3%) 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. Neurobehavioral data on Yu-Cheng children obtained after 1985 show that the intellectual development of these children continues to lag somewhat behind that of matched control children. As measured from 1985 through 1990, this effect persists at least up to the age of 7 years, and it occurs in children born long after the initial Yu-Cheng exposure. Also, Yu-Cheng children are rated by their parents and teachers to have a higher activity level, more health, habit, and behavioral problems, and to have a temperament 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 vs. nonTCDD-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 vs. non-TCDD-like congeners in causing this toxicity can be understood. Mechanisms that respond uniquely to TCDD-like congeners may not necessarily be involved inasmuch as three lightly chlorinated, ortho-substituted PCB congeners, 2,4,4'-TCB, 2,2',4,4'-TCB, and 2,2',5,5'-TCB, were 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. 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.

D. 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 8, birds in Table 9, and mammals in Table 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 blue bird eggs were injected directly with the indicated doses of TCDD. Thus, the doses of TCDD given in Tables 8 and 9 for all fish and bird species represent TCDD egg burdens in which 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 10 are maternal TCDD doses in which 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 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 elimination half-life for TCDD in rats. Therefore, the body burden of TCDD given for the rat is 92.8% of the calculated steady-state body burden. In rhesus monkeys, the half-life for whole body elimination of TCDD is longer than was 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

<table>
<thead>
<tr>
<th>Species</th>
<th>Effect</th>
<th>Exposure</th>
<th>Egg Dose (ng/kg)</th>
<th>Effect level</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake trout</td>
<td>Sac fry mortality</td>
<td>Static waterborne</td>
<td>34</td>
<td>NOAE L</td>
<td>14</td>
</tr>
<tr>
<td>Japanese medaka</td>
<td>Lesions*</td>
<td>Static waterborne</td>
<td>&lt;100</td>
<td>NOAE L</td>
<td>22</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Sac fry mortality</td>
<td>Single injection</td>
<td>194</td>
<td>NOAE L</td>
<td>275</td>
</tr>
<tr>
<td>Lake trout</td>
<td>Sac fry mortality</td>
<td>Static waterborne</td>
<td>40</td>
<td>LOAE L</td>
<td>23</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Sac fry mortality</td>
<td>Static waterborne</td>
<td>55</td>
<td>LOAE L</td>
<td>14</td>
</tr>
<tr>
<td>Japanese medaka</td>
<td>Sac fry mortality</td>
<td>Single injection</td>
<td>291</td>
<td>LOAE L</td>
<td>275</td>
</tr>
<tr>
<td></td>
<td>Lesions*</td>
<td>Static waterborne</td>
<td>300</td>
<td>LOAE L</td>
<td>22</td>
</tr>
</tbody>
</table>

* 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 9
Cross-Species Comparison of NOAELS and LOAELS for TCDD Developmental Toxicity in Birds

<table>
<thead>
<tr>
<th>Species</th>
<th>Effect</th>
<th>Exposure</th>
<th>Egg Dose (ng/kg)</th>
<th>Effect level</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ring-necked pheasant</td>
<td>Embryo mortality</td>
<td>Single injection</td>
<td>100</td>
<td>NOAEL</td>
<td>31</td>
</tr>
<tr>
<td>Eastern bluebird</td>
<td>Embryo mortality</td>
<td>Single injection</td>
<td>1,000</td>
<td>NOAEL</td>
<td>41</td>
</tr>
<tr>
<td>Chicken</td>
<td>Cardiac malformations</td>
<td>Single injection</td>
<td>9a</td>
<td>LOAEL</td>
<td>32</td>
</tr>
<tr>
<td>Chicken</td>
<td>Embryo mortality</td>
<td>Single injection</td>
<td>240</td>
<td>LD_{50}</td>
<td>30</td>
</tr>
<tr>
<td>Ring-necked pheasant</td>
<td>Embryo mortality</td>
<td>Single injection</td>
<td>1,000</td>
<td>LOAEL</td>
<td>31</td>
</tr>
<tr>
<td>Ring-necked pheasant</td>
<td>Embryo mortality</td>
<td>Single injection</td>
<td>1,354a</td>
<td>LD_{50}</td>
<td>31</td>
</tr>
<tr>
<td>Ring-necked pheasant</td>
<td>Embryo mortality</td>
<td>Single injection</td>
<td>2,182a</td>
<td>LD_{50}</td>
<td>31</td>
</tr>
<tr>
<td>Eastern bluebird</td>
<td>Embryo mortality</td>
<td>Single injection</td>
<td>10,000</td>
<td>LOAEL</td>
<td>41</td>
</tr>
</tbody>
</table>

a Chi-square analysis of the data in Table 1 of Cheung et al. 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.

intake are approximately 3 times greater than the maternal body burdens estimated at the time of conception (Table 10). Both in rats and rhesus monkeys, the maternal body burdens are calculated using a one-compartment open model, assuming 86.1% bioavailability for TCDD. The bioavailability used for TCDD was determined in rats. Because no estimate for TCDD bioavailability has been reported in rhesus monkeys, the same 86.1% value was used. The whole body elimination half-life used for TCDD in the rat is 23.7 days. McNulty et al. estimated a half-life of approximately 1 year for TCDD elimination from adipose tissue in the rhesus monkey; for calculation of the body burdens estimated in Table 9, 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. The maternal body burdens given for subchronic exposure in the rhesus monkey were calculated from data obtained from Bowman, which included the daily dietary TCDD exposure level for each pregnant female used in the studies reported by Bowman et al. and Schantz and Bowman. 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. 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 8, lake trout and rainbow trout sac fry and Japanese medaka embryos are similarly affected by a spectrum of lesions, which includes hemorrhage, edema, collapse of the yolk sac, cessation of blood flow, and embryo mortality. Estimates of the NOAEL and LOAEL are given in the table for the appearance of these lesions in Japanese medaka embryos and for embryo mortality in the two trout species. 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; however, the egg doses given in Table 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 the method of TCDD exposure. Of the three fish species, lake trout sac fry are the most sensitive to TCDD-induced mortality.
ever, based on the LOAELs shown in Table 8, the difference in susceptibility between fish species may be less than tenfold.

Based on the LOAELs shown in Table 9, the sensitivity of different bird species to TCDD-induced embryo mortality varies more than 40-fold. The chicken embryo is more susceptible to TCDD-induced mortality than are embryos of the ring-necked pheasant and eastern blue bird. 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 examined.

Table 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 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 10 indicate that the embryo/fetus of rodent species is generally not as sensitive to TCDD-

<table>
<thead>
<tr>
<th>Species</th>
<th>Effect</th>
<th>Exposure</th>
<th>Maternal dose</th>
<th>Effect level</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey</td>
<td>Prenatal mortality</td>
<td>Multiple dose</td>
<td>22 ng/kg, 9 ×, gd 20–40</td>
<td>NOAEL 74</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>Prenatal mortality</td>
<td>1 ng/kg/day</td>
<td>27 ng/kg,* chronic</td>
<td>NOAEL 228</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>Prenatal mortality</td>
<td>Multiple dose</td>
<td>30 ng/kg/day, gd 6–15</td>
<td>NOAEL 62</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>Hydronephrosis</td>
<td>Multiple dose</td>
<td>100 ng/kg/day, gd 6–15</td>
<td>NOAEL 276</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>Cleft palate</td>
<td>Multiple dose</td>
<td>300 ng/kg/day, gd 6–15</td>
<td>NOAEL 59</td>
<td></td>
</tr>
<tr>
<td>Monkey</td>
<td>Object learning</td>
<td>0.126 ng/kg/day</td>
<td>19 ng/kg,* subchronic</td>
<td>LOAEL 69</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>Male reproductive</td>
<td>Single dose</td>
<td>64 ng/kg, gd 15</td>
<td>LOAEL 163–165</td>
<td></td>
</tr>
<tr>
<td>Monkey</td>
<td>Prenatal mortality</td>
<td>0.642 ng/kg/day</td>
<td>97 ng/kg,* subchronic</td>
<td>LOAEL 69</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>Extra ribs</td>
<td>Multiple dose</td>
<td>111 ng/kg, 9 ×, gd 20–40</td>
<td>LOAEL 74</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>Fetal growth</td>
<td>Multiple dose</td>
<td>100 ng/kg/day, gd 6–15</td>
<td>LOAEL 79</td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>Prenatal mortality</td>
<td>Multiple dose</td>
<td>125 ng/kg/day, gd 6–15</td>
<td>LOAEL 62</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>Prenatal mortality</td>
<td>10 ng/kg/day</td>
<td>250 ng/kg/day, gd 6–15</td>
<td>LOAEL 79</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>Prenatal mortality</td>
<td>Multiple dose</td>
<td>270 ng/kg,* chronic</td>
<td>LOAEL 228</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>Hydronephrosis</td>
<td>Multiple dose</td>
<td>50 ng/kg/day, gd 6–15</td>
<td>LOAEL 62</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>Prenatal mortality</td>
<td>Single dose</td>
<td>500 ng/kg/day, gd 6–15</td>
<td>LOAEL 149</td>
<td></td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Prenatal mortality</td>
<td>Single dose</td>
<td>1,500 ng/kg, gd 14</td>
<td>LOAEL 57</td>
<td></td>
</tr>
<tr>
<td>Hamster</td>
<td>Thymic hypoplasia</td>
<td>Single dose</td>
<td>1,500 ng/kg, gd 7 or 9</td>
<td>LOAEL 57</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>Cleft palate</td>
<td>Multiple dose</td>
<td>3,000 ng/kg/day, gd 6–15</td>
<td>LOAEL 77</td>
<td></td>
</tr>
<tr>
<td>Hamster</td>
<td>Prenatal mortality</td>
<td>Single dose</td>
<td>18,000 ng/kg/day, gd 7 or 9</td>
<td>LOAEL 61</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>Prenatal mortality</td>
<td>Single dose</td>
<td>24,000 ng/kg/day, gd 6</td>
<td>LOAEL 60</td>
<td></td>
</tr>
</tbody>
</table>

Note: gd = gestational day.

* Maternal body burdens of TCDD at the time of conception were calculated by assuming a one-compartment open model and a half-life for whole body TCDD elimination of 400 days in the monkey\textsuperscript{27} and 23.7 days in the rat\textsuperscript{28} with a bioavailability of 86.1% was used in the monkey and rat\textsuperscript{29} The daily dietary exposure levels in rhesus monkeys were approximately 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 accumulated amount of TCDD in rhesus monkeys was 30.5% 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 accumulated amount of TCDD in rats was 92.8% of the calculated steady-state level.
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 1000- to 5000-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 9 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 agrees with the maternal TCDD body burden of 270 ng/kg calculated from the chronic exposure to within a factor of 2. Similarly, the TCDD dose of 111 ng/kg that was given to rhesus monkeys 9 times during the first trimester of pregnancy agrees with the maternal body burden of 97 ng/kg that increased prenatal mortality in rhesus monkeys following subchronic dietary exposure.

III. REPRODUCTIVE TOXICITY

A. Female

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 next. Other TCDD effects on pregnancy maintenance, embryo/fetotoxicity, and postnatal development were covered in Section II.

The study by Murray et al. used a multi-generational approach to examine the reproductive effects of exposure of male and female rats over three generations to relatively low levels of TCDD (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. Even so, 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 F₀ generation and at 0.01 μg/kg/day in the F₁ and F₂ generations. Additionally, in a 13-week exposure to 1 to 2 μg/kg/day of TCDD in non-pregnant female rats, Kociba et al. 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. reported no effects on the female reproductive system.

Allen and colleagues reported on the effects of TCDD on reproduction in the monkey. 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. 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. One monkey delivered a stillborn infant and only one conception resulted in a live birth. As described in an abstracted summary, these results at 50 and 500 ppt TCDD were compared to 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. 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 50 ppt TCDD, there were no overt effects on maternal health, but the ability to conceive and maintain pregnancy was reduced.

In a similar series of experiments, female rhesus monkeys were fed diets that contained 0, 5, and 25 ppt TCDD. Reproductive function was not altered in the 5 ppt group since, after 7 months of dietary exposure to TCDD, seven of
eight females mated to unexposed males 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 gave 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 fetal mortality without producing overt toxic effects in the mother.

McNulty\textsuperscript{74} 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 different from the control population, but the low number of animals per group did not permit statistical analysis. McNulty\textsuperscript{74} also administered single 1-µg/kg doses of TCDD on gestational day 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, for 16 monkeys given 1 µg TCDD/kg in single or divided doses between days 25 and 40 of pregnancy, there were only 3 normal births.\textsuperscript{65,74}

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 a few studies, some signs of ovarian dysfunction, such as anovulation and suppression of the estrous cycle, have been reported.\textsuperscript{71,72,230} 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 about how TCDD toxicity involving the mother and/or placenta may affect fetal development.

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 \(\leq 9\) months, the length of the menstrual cycle and the intensity and duration of menstruation were not appreciably affected by TCDD exposure.\textsuperscript{72} However, there was a decrease in serum estradiol and progesterone concentrations 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.\textsuperscript{71} 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 the serum estradiol and progesterone concentrations of these monkeys. Thus, the presence of these hormonal alterations is dependent on the level of dietary TCDD exposure. Shiverick and Muther\textsuperscript{732} reported that there was no change in circulating levels of estradiol in the rat after exposure to 1 µg/kg/day TCDD 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, although more research needs to be done to examine the effects of TCDD on female hormones.

3. Antiestrogenic Action

a. In Vivo

Estrogens are necessary for normal uterine development and for maintenance of the adult
uteros. 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 indicated that chronic exposure to TCDD can cause suppression or inhibition of the estrous cycle. Rhesus monkeys chronically exposed to TCDD, on the other hand, are affected by hormonal irregularities in their menstrual cycles. Although the authors of these studies in rats and monkeys did not attribute the effects of TCDD exposure to an antiestrogenic action, it seems reasonable to posit from more recent information that such an antiestrogenic action may have been responsible.

In rhesus monkeys, the severity of the TCDD-associated reproductive alterations was correlated with decreased plasma levels of estrogen and progesterone. 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. 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. 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, a decrease in uterine peroxidase activity, and a decrease in the concentration of progesterone receptors in the uterus. 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. Similarly in mice, TCDD administration decreases uterine weight and antagonizes the ability of 17β-estradiol to increase uterine weight.

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 of progesterone receptor concentration. On the other hand, in 28-day-old intact rats and 70-day-old ovariec-tomized rats, both of these 17β-estradiol-mediated responses are attenuated by TCDD. Previously, it was reported that TCDD administration does not alter the dose-dependent increase in uterine weight due to exogenously administered estrone in sexually immature rats. The later work by Safe et al. suggest that this apparent lack of an antiestrogenic effect of TCDD may have been due to the young age of the rats used.

b. In Vitro

Both TCDD and progesterone can cause a decrease in the nuclear estrogen receptor concentration in rat uterine strips. 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 why the TCDD-induced decrease in nuclear estrogen receptors is blocked by a transcription inhibitor, but not by protein synthesis inhibitors, are not known. However, these results indicate that TCDD and progesterone decrease the nuclear estrogen receptor concentration via different mechanisms. In addition, the antiestrogenic actions of TCDD can be demonstrated in cell culture, and two prominent mechanisms could potentially be involved: (1) increased me-
tabolism 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-, 52-, and 34-kDa protein.239 The 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 tissue plasminogen activator (tPA) and inhibits estrogen-dependent postconfluent cell proliferation.240,241 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 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. 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.243 TCDD treatment also results in reduced levels of occupied nuclear estrogen receptors.244 In MCF-7 cells, these results indicate that the antiestrogenic effect of TCDD could result from (1) the increased metabolism of estrogens due to Ah receptor-mediated enzyme induction, and/or (2) a decreased number of estrogen receptors in the nucleus. Safe’s group has published TCDD-concentration response information for both the TCDD-induced decrease in occupied nuclear estrogen receptors245 and the induction of AHH and EROD activities in MCF-7 cells.246 In addition, they have reported that TCDD causes a decreased number of cytosolic and nuclear estrogen receptors in Hepa 1c1c7 cells, which is a mouse hepatoma cell line.246 Independent analysis of the data suggests that the EC50 values for these effects are not dissimilar 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 reported that this concentration is the approximate EC50 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’s group used an analog of TCDD, i.e., MCDF, that 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 for which there was no detectable induction of EROD activity.248 In addition, it has been stated that the downregulation of estrogen receptors in Hepa 1c1c7 cells can be detected as early as 1 h after exposure of the cell cultures to 10 nM TCDD.246 This time is slightly less than the 2 h required by Israel and Whitlock247 to detect an increase in cytochrome P-4501A1 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-4501A1 mRNA after 1 h, whereas the induction of AHH activity takes slightly longer.247

Gierthy et al.240 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. 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.,240 which suggests no effect of TCDD on the estrogen receptor content of MCF-7 cells, and the contrary results of Safe’s group in this same cell line remains largely unex-
plained. 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 the 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.238,249

c. Evidence for an Ah Receptor Mechanism

i. Ah Receptor Mutants

Although the antiestrogenic effects of TCDD may be caused by either a decreased number of occupied nuclear estrogen receptors and/or the increased metabolism of estrogen, there is evidence that the antiestrogenic effect of TCDD 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.246 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-450IA1 mRNA transcription and enzyme activity in Hepa 1c1c7 cells.247 This effect also is Ah receptor-mediated.27

ii. Structure-Activity Relationships In Vivo

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.250 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-PCDD 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%, whereas uterine estrogen receptor concentrations are reduced 53 and 49% by TCDD and 1,2,3,7,8-PCDD, respectively. On the other hand, 1,3,7,8-TCDD and 1,2,4,7,8-PCDD 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%, whereas uterine estrogen receptor concentrations are reduced 21 and 24% by 1,3,7,8-TCDD and 1,2,4,7,8-PCDD, respectively.249 Because 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.

iii. Genetic Evidence

Consistent with the interpretation based on structure-activity relationships, there is a greater reduction in the number of hepatic estrogen receptors when AhbAhb C57BL/6 mice are exposed to TCDD than when AhbAhb DBA/2 mice are similarly exposed.251 To date, however, the antiestrogenic effects have not been studied in the progeny of test crosses between AhbAhb and AhbAhb 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.

iv. 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.254 The relative abilities of TCDD and other CDD, CDF, and PCB con-
curs perinatally rather than in adulthood. To illustrate this sensitivity, a single maternal TCDD dose of 0.16 μg/kg given on day 15 of gestation affects several endpoints in male offspring that collectively indicate a deficit in androgenic status. These include decreases in the weights of accessory sex organs such as the ventral prostate and epididymis and cauda epididymis, as well as a decreased anogenital distance and increased time of testis descent. In adult male rats exposed perinatally to TCDD, the effects on sexual behavior included increases in the number of mounts preceding ejaculation and intromission latency. These effects were produced by single TCDD doses as low as 0.064 μg/kg given on day 15 of gestation. When exposure to TCDD occurs in adulthood on the other hand, relatively large doses in the overtly toxic range are required to cause decreases in ventral prostate and caput epididymis weight. Kociba et al. reported that accessory sex organ weights 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., this dosage regimen results in a TCDD body burden of approximately 20 μg/kg at the end of the dosing period. This body burden is similar to the ED₅₀ of 15 μg/kg determined by Moore et al. for some adverse effects of TCDD, including decreased plasma androgen concentrations and accessory sex organ weights in adult male rats. In addition, it is at least 100 times greater than doses of TCDD that decreased androgenic status after perinatal exposure.

In male rats, TCDD exposure results in decreased spermatogenesis. This effect occurs after exposure in adulthood to single doses of TCDD as low as 3 μg/kg, whereas male rats exposed perinatally to only 0.064 μg TCDD/kg given on day 15 of gestation are similarly affected in adulthood. However, a comparison of the TCDD doses in adult rats that decrease plasma testosterone levels and accessory sex organ weights with that which decreases spermatogenesis suggests that decreases in plasma androgen concentrations and/or androgen responsiveness in TCDD-treated adult male rats may not completely explain the effects of TCDD on spermatogenesis. Similarly, the reduction in plasma testosterone concentration in perinatally exposed male rats may be insufficient to explain the effects of TCDD exposure on spermatogenesis.

In adult rats, the most sensitive toxic responses to TCDD have been observed following long-term, low-level exposure. In a three-generation reproduction study, Murray et al. 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. Based on the pharmacokinetics of TCDD in the rat, 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. found that a single TCDD dose of 0.064 μg/kg given on day 15 of gestation produced 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, defined as the lowest dose used that had no statistically significant effect, could not be determined by Mably et al. It is concluded that developmental effects on spermatogenesis occur at a maternal TCDD dose that is lower than any dose previously shown to produce toxicity in rats.

IV. SUMMARY

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. A substantial portion of the literature reviewed was published after 1985 and a special effort has been made to view the developmental toxicity of TCDD in light of the Ah receptor model of TCDD. On the other hand,
there is very little, if any, information available that can be used to relate the reproductive toxicity of TCDD to this receptor model.

We chose to structure this review into sections on developmental toxicity and male and female reproductive toxicity, but we recognize and want to emphasize that developmental and reproductive events are interrelated at all levels of biological complexity. Therefore, the reader should not view the section subheadings within each of these divisions as defining discrete endpoints that are exclusive of other endpoints. For example, effects of TCDD on circulating levels of sex hormones and/or on responsiveness to sex hormones may be translated into reproductive dysfunction if exposure occurs in adulthood as well as 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 inhibition of prenatal growth can significantly disrupt the structural integrity of an organ system.

Given the current data base, developmental toxicity endpoints tend to be observed at lower TCDD exposure levels than are endpoints of male and female reproductive toxicity. The lowest effective TCDD egg burden for causing developmental toxicity in fish and birds and the lowest effective maternal TCDD body burden for producing a wide range of developmental responses in mammals are summarized in Tables 8, 9, and 10, respectively. These results indicate that a wide variety of developmental events, crossing three vertebrate classes and several species within each class, can be perturbed, thus suggesting that TCDD has the potential to disrupt a large number of critical developmental events at specific developmental stages. In addition, only transient exposure to relatively low levels of TCDD at critical times may be all that is necessary to cause irreversible disruptions in organ system or function. Higher TCDD exposure levels cause embryo/fetal mortality, and this lethal effect of TCDD-like congeners clearly poses a danger to populations of the most sensitive native fish and wildlife species.

Because developmental toxicity following exposure to TCDD-like congeners occurs in fish, birds, and mammals, it is likely to occur in man. Certain effects in human infants exposed to a complex mixture of PCBs, CDFs, and PCQs in the Yusho and Yu-Cheng poisoning episodes were probably caused by the combined exposure to those PCB and CDF congeners that are Ah receptor agonists. The effects observed in human infants perinatally exposed to this complex mixture were similar to those reported in newborn mice and adult monkeys exposed only to TCDD, and this comparability of effects increases the probability that the outcomes of exposure in Yusho and Yu-Cheng children were due to the TCDD-like congeners in the contaminated rice oil ingested by their mothers. Most significant is a clustering of effects in organs derived from ectoderm, a syndrome referred to as ectodermal dysplasia. Included in this syndrome are effects on the skin, nails, and meibomian glands that have occurred in both adult monkeys exposed to TCDD and in the Yusho and Yu-Cheng infants exposed transplacentally to PCB-, CDF-, and PCQ-contaminated rice oils. In addition, accelerated tooth eruption has been reported both in human infants affected by the Yusho and Yu-Cheng exposures and in neonatal mice exposed to TCDD. The CNS also is derived from ectoderm, and the occurrence of functional neurotoxic effects due to perinatal TCDD exposure in monkeys and rats suggests that brain function as a site of action of TCDD may be involved in the ectodermal dysplasia syndrome. Yu-Cheng children transplacentally exposed to PCB-, CDF-, and PCQ-contaminated rice oil have produced a clinical impression of developmental delay and psychomotor delay during developmental and cognitive tests, whereas monkeys perinatally exposed to TCDD are affected by a deficit in cognitive function. The concept that the ectodermal dysplasia syndrome in the Yusho and Yu-Cheng infants may be caused by the combination of PCB and CDF congeners in the rice oil that are Ah receptor agonists, but are less potent that TCDD, is consistent with structure-activity results for various developmental endpoints in different species of fish, birds, and mammals. These studies indicate that while there is variability between species in the profile of developmental responses elicited by TCDD, essentially all TCDD-like PCB, CDD, and CDF congeners that have Ah receptor affinity and intrinsic activity produce the
same pattern of developmental effects within a given vertebrate species if a sufficiently high dose of the congener is given.

In mammals, postnatal functional alterations involving learning behavior and the developing male reproductive system appear to be the developmental events most sensitive to perinatal dioxin exposure. A maternal TCDD body burden of 19 ng/kg in the rhesus monkey at the time of conception is associated with impaired object learning in the offspring when they are tested at approximately 14 months of age. In the rat, a single maternal dose of TCDD as low as 64 ng/kg on day 15 of gestation results in a reduction in spermatogenesis and alteration in masculine sexual behavior of the male offspring in adulthood. In birds and fish, structural malformations and embryo mortality are the most sensitive effects observed after direct injection of TCDD into fertilized eggs. In chicken embryos, a 17% increase in cardiovascular malformations was produced by an egg TCDD dose as low as 9 ng/kg. In lake trout sac fry, the lowest TCDD egg burden to increase mortality was 40 and 55 ng/kg/egg.

In mammals, alterations in structural endpoints and diminished prenatal viability and growth begin to predominate during gestation at maternal TCDD body burdens and/or daily TCDD doses that are above 100 ng/kg. The incidence of a structural malformation that consists of extra ribs in rabbits is increased at a maternal TCDD dose during gestation of 100 ng/kg/day. Prenatal mortality in monkeys has been observed where the maternal body burden at the onset of pregnancy is estimated to be 111 ng/kg, a level that produces no signs of overt maternal toxicity. In rats, fetal growth is inhibited at a maternal TCDD dose of 125 ng/kg/day during gestation. In mice, hydronephrosis can be elicited at a maternal TCDD dose of 500 ng/kg/day administered on gestational days 6 to 15, and in hamsters the same response can be caused by a single 1500 ng/kg dose of TCDD administered to the dam on each gestational day 7 or 9. These doses of TCDD that cause hydronephrosis in mice and hamsters are not maternally toxic. In the mouse, cleft palate is produced at a maternal TCDD dose of 3000 ng/kg/day administered on gestational days 6 to 15. Prenatal mortality in the mouse results from a single maternal dose of 24,000 ng/kg administered on gestational day 6, however, larger doses of TCDD are required to produce prenatal mortality in the mouse when administered on later gestational days. A general finding in fish, bird, and mammalian species is that the embryo or fetus is more sensitive to TCDD-induced mortality than the adult. Thus, the timing of TCDD exposure during the life history of an animal can greatly influence its susceptibility to overt dioxin toxicity.

With respect to male and female reproductive endpoints, there are clear effects following dioxin exposure of the adult animal. Such reproductive effects generally occur at TCDD body burdens that are higher than those required to cause the more sensitive developmental endpoints. For example, TCDD exposure of the adult male causes reduced testis and accessory sex organ weights, abnormal testis structure, decreased spermatogenesis, reduced fertility, decreased testicular testosterone synthesis, reduced plasma androgen concentrations, and altered regulation of pituitary LH secretion. However, these effects only appear at TCDD exposure levels that are overtly toxic to the animal. In the more limited studies focusing on female reproduction, the primary effects include decreased fertility, inability to maintain pregnancy, and, in the rat, decreased litter size. Signs of ovarian dysfunction and alterations in hormone levels also have been reported.

Exposure of female mice and rats to TCDD has an antiestrogenic effect. The dose of TCDD required to produce this response is generally higher than that needed to cause the most sensitive signs of developmental toxicity in these species. More specifically, hydronephrosis and cleft palate in mice and reductions in spermatogenesis in rats occur at maternal doses of TCDD that are far less than those needed to exert an antiestrogenic effect when young adult female mice and rats are exposed to dioxin. The precise mechanism of the antiestrogenic effect of TCDD is not fully understood. It may be caused by both a decrease in estrogen receptor number and/or by an increase in cytochrome P-4501A1-mediated estrogen metabolism within the target cell. The antiestrogenic action of TCDD is significant in that it may provide insight not only into the cause of certain female reproductive effects, but also developmental effects, such as the feminization of sexual behavior in male rats.
The most convincing evidence for establishing a role of the Ah receptor in causing a particular TCDD endpoint is to show that genetic linkage exists between the expression of that endpoint and a particular allele at the Ah locus. Of all the developmental and reproductive effects of TCDD, such genetic linkage has been demonstrated only for structural malformations in mice and antiestrogenicity in Hepa 1c1c7 cells. Structure-activity relationships can be used to support genetic evidence where it exists, or in the absence of genetic evidence, to indicate a probable role for Ah receptor involvement. The very limited amount of structure-activity type evidence available for embryo/fetal mortality due to TCDD-like congeners in fish, birds, and mammals suggests that this effect also may involve the Ah receptor. For other developmental and reproductive effects of TCDD, there have been no genetic assessments or relative potency evaluations of congeners for Ah receptor involvement. Although it remains possible that an Ah receptor mechanism will eventually be demonstrated for many more of these effects, it also is feasible that the Ah receptor mechanism may not be involved in all of them.

ACKNOWLEDGMENTS

The authors acknowledge Dr. Barbara D. Abbott, Dr. Linda S. Birnbaum, Mr. Donald L. Bjerke, Dr. Peter L. Defur, Dr. John F. Gierthy, Dr. Claude L. Hughes, Dr. Robert W. Moore, Dr. Stephen Safe, Dr. Susan L. Schantz, and Dr. Hugh M. Tilson for reviewing an earlier version of this publication. This work was supported in part by NIH grant ESO1332.

DISCLAIMER

The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency. The U.S. Government has the right to retain a nonexclusive royalty-free license to any copyright covering this article.

REFERENCES

1. Safe, S., 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), CRC Crit. Rev. Toxicol., 21, 51, 1990.


34. Brunstrom, B. and Barnerud, P. O., Toxicity and distribution in chick embryos of 3,3′,4,4′-tetrachlorobiphenyl injected into the eggs, *Toxicology*, 27, 103, 1983.


67. Khera, K. S., Extraembryonic tissue changes induced by 2,3,7,8-tetrachlorodibenzo-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, 1992.


69. Schantz, S. L. and Bowman, R. E., Learning in monkeys exposed perinatally to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), Neurotoxicol. Teratol., 11, 13, 1989.


92. Rogan, W. J., Gladem, B. C., Hung, K.-L., Koong, S.-L., Shih, L.-Y., Taylor, J. S., Wu, Y.-C., Yang, D., Ragan, N. B., and Hsu, C.-C., Congenital poi-


232. Shiverick, K. T. and Muther, T. F., 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) effects on hepatic


246. Zacharewski, T., Harris, M., and Safe, S., Evidence for the 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, 10390, 1983.


