Postnatal Effects of Maternal Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)

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Previous studies reported that subcutaneous administration of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at a dose level of 3 μg/kg, in mice on days 6 through 15 of gestation produced pups with cleft palates and kidney anomalies (*1*). The C57B1/6 mouse was the most sensitive of the three strains tested to the TCDD-induced kidney effects, in that almost 100% of the fetuses developed kidney anomalies. The purpose of this paper is to report subsequent studies which attempt to characterize the teratogenic response as seen in the C57B1/6 mouse with specific emphasis on the nature and significance of the kidney anomaly. To distinguish between delayed kidney development, which if transient is of diminished significance, and irreversible effects, postnatal studies were also conducted. Reversible delays in metanephric kidney maturation in the rat have been described (*2*).

Materials and Methods

Inbred mice of the C57B1/6 strain were obtained from either the Jackson Laboratory, Bar Harbor, Maine, or the AR Schmidt Company, Madison, Wisconsin, and mated at the Institute. Detection of a vaginal plug indicated day 0 of pregnancy. All experimental mice were singly housed in plastic cages and allowed free access to food and water.

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The 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (>99% purity, Dow Chemical Company, Midland, Michigan) was dissolved in acetone and subsequently diluted with at least 9 parts of corn oil. All mice were weighed prior to dosing, and the oral dose administered computed on the mean weight of the mice being treated. Control mice received an equivalent amount of 0.1 ml of an acetone–corn oil preparation.

Fetuses were removed from their mother on gestation day 18 and necropsied after fixation in Bouins solution. Mice necropsied in the postnatal studies were processed according to standard necropsy procedures. Tissues for histologic examination were fixed in either Bouins or 10% neutral buffered formalin, paraffin embedded and stained with hematoxylin and eosin. The incidence of an abnormality is given as mean average percent which is derived by determining its percent incidence in a litter and subsequently computing the mean of these percents.

Results

The effects of maternal treatment with TCDD on fetal palate closure and kidney development are shown in Table 1. TCDD at 3 μg/kg, administered on gestation days 10 through 13, produced cleft palate in pups from 12 of 14 litters with a mean average incidence of 55.4%. The mean average kidney and bilateral kidney incidence was 95.1% and 83.1%, respectively, with all lit-

ters affected. When the dose of TCDD administered on gestation days 10 through 13 was reduced to 1 μg/kg, cleft palate incidence decreased to a mean of 1.9%. The litter incidence of kidney anomalies persisted at high levels; unilateral mean average pup incidence decreased to 58.9% and the corresponding figure for bilateral effect decreased to 36.3%. When TCDD administration at 1 μg/kg was a single dose administered on gestation day 10, no cleft palates were produced. The average mean incidence of unilateral kidney effects was 34.3% with anomalies occurring in 16 of 18 litters. Bilateral kidney effects at this dose occurred in seven litters with a mean incidence of 8.8%. No cleft palates or kidney anomalies occurred in controls.

The fetal kidney anomaly is best described as a renal papilla which is markedly reduced in size, or nonexistent in a few cases, resulting in an enlarged renal pelvis (Fig. 1). Given the stage of kidney development this may reflect a retardation or absence of papillae development rather than loss of an already formed structure. Nephron development appears similar to that occurring in control mice of the same age. The appearance of the affected kidneys resemble an early stage of hydronephrosis. It was further noted that when the renal anomalies were unilateral, the right kidney was affected 70.5% of the time. This preponderance of right kidney involvement had a high statistical significance ($P<0.01$).

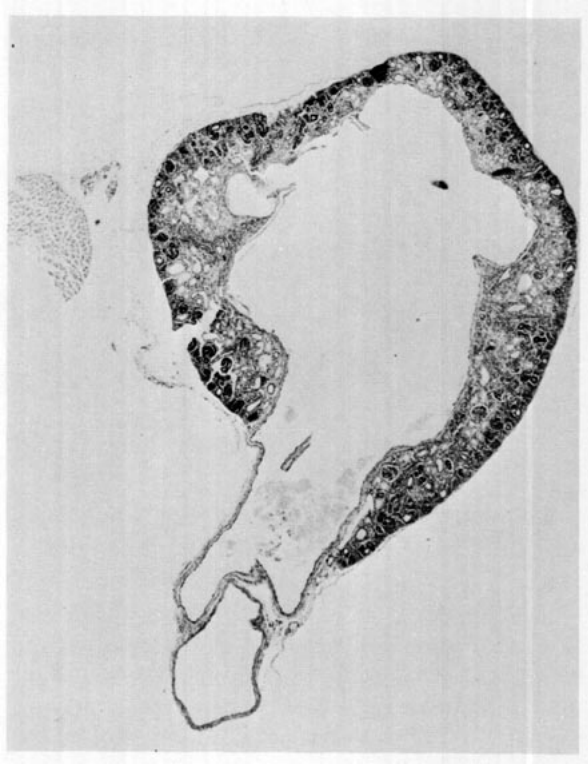

FIGURE 1. Transverse section of a hydronephrotic kidney from an 18-day-old C57B1/6 fetus whose mother received 3 μg/kg TCDD on gestation days 10–13.

To assess the impact of this kidney change on the ability of a pup to survive in an extrauterine environment, postnatal studies were conducted. Pregnant C57B1/6 mice received 1 μg/kg TCDD on gestation day 10 and were allowed to litter. In one study litters from TCDD-treated mothers were fos-

Table 1. Incidence of cleft palate and kidney anomalies in C57B1/6 fetuses from TCDD-treated mothers.[a]

			Cleft palate		Kidney anomalies		Bilateral kidney anomalies	
Treatment days	TCDD dose, μg/kg	No. litters	No. affected litters	Mean average %	No. affected litters	Mean average %	No. affected litters	Mean average %
10–13	3	14	12	55.4	14	95.1	14	83.1
10–13	1	16	3	1.9	15	58.9	13	36.3
10	1	18	0	0	16	34.3	7	8.8
10, 10–13	0	27	0	0	0	0	0	0

[a] No significant differences in the number of live fetuses, resorptions, fetal weight and maternal weight was found at these dose levels when compared to controls.

tered on control mothers; litters from control mice nursed TCDD treated mice. To distinguish a prenatal effect from a postnatal effect or from a combined prenatal and postnatal effect, a reciprocal cross fostering study was conducted. The results of these studies as they affected kidney are depicted in Table 2. It was found that only one pup from a TCDD-treated mother, who nursed an untreated mother, had kidney lesions when necropsied on postnatal day 14. A total of six pups, in four of the 14 litters, who nursed TCDD mothers but were born of untreated mothers had hydronephrotic kidneys. In the four-way cross foster study, almost all kidney lesions occurred in pups whose mother received 1 μg/kg TCDD and subsequently nursed a TCDD treated mother. Five of 7 litters contained pups with hydronephrotic kidneys with a mean average pup incidence of 34%. Almost one half of the affected pups had both kidneys affected. Three pups in two litters, from untreated mothers who nursed a TCDD-treated mother, also had kidney anomalies; one was bilaterally involved. One pup from a TCCD-treated female which nursed an untreated mother had an affected kidney, as did one of the control pups.

To confirm that exposure to TCDD-treated females during the nursing period was a major factor in development of renal hydronephrosis in mouse pups, experiments where mothers were treated on the day of parturition were conducted. In these studies, mice received 0, 1, 3 or 10 μg/kg at parturition developed hydronephrosis. A mean average of 75% of the pups developed at least unilateral hydronephrosis; a mean average of 40% developed bilateral hydronephrosis. At the 3 μg/kg dose, a mean average of 71% of pups in three of three litters developed a right unilateral hydronephrosis. At the 1 μg/kg dose level, a mean average of 12% of pups in five of nine litters developed a unilateral hydronephrosis.

Gross and histological studies of affected mice reveal that the lesion produced is a progressive hydronephrosis. Essentially, total atrophy of the right kidney had occurred in several 55-day-old mice which had nursed a female that received 10 μg/kg TCDD at parturition (Fig. 2). There were

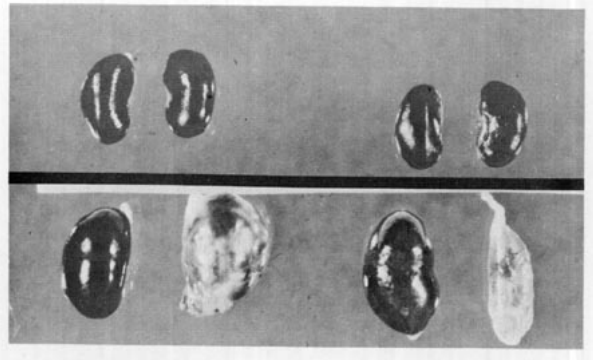

FIGURE 2. Gross photograph of kidneys from 55-day-old C57B1/6 mice: (top row) left and right kidneys from mice who nursed a control mother. (bottom row) kidneys from mice who nursed a mother treated with 10 μg/kg TCDD. Left kidney enlarged and fluid-filled. Right kidneys with marked hydronephrosis and complete atrophy of renal parenchyma (lower right).

Table 2. Incidence of hydronephrosis in C57B1/6 mouse pups following exposure to TCDD; mice treated at 1 μg/kg on day 10 of pregnancy.[a]

Period exposed to TCDD mother			Kidney hydronephrosis		Bilateral hydronephrosis	
In utero	Postnatal	No. litters	No. affected litters	Mean average %	No. affected litters	Mean average %
Yes	No	14	1	2.0	0	0
No	Yes	14	4	9.6	1	0.8
Yes	Yes	7	5	34.0	4	17.0
Yes	No	5	1	2.2	0	0
No	Yes	5	2	7.4	1	0.5
No	No	7	1	1.7	0	0

[a] No significant differences in pup weight, maternal weight or litter mortality was observed.

never any indications of a hydroureter accompanying the hydronephrosis. Serial sections of some renal pelves at the site of ureter entrance did not indicate a lack of patency. Serial sections at the site of ureter insertion into the bladder also indicated that there was no blockage. There is a weak impression that the ureter associated with a hydronephrotic kidney has a lumen of smaller diameter and that there may be a slight thickening or increase in the epithelial cells of the ureter mucosa. In those mice in which unilateral renal hydronephrosis occurred, the right kidney was affected over 90% of the time. The tendency for unilateral hydronephrosis to involve the right kidney suggests an anatomical variation which predisposes its involvement at low dose levels. The right kidney in mice is normally superior in position; the vena cava, due to its close proximity to the right kidney, provides less room for ureter insertion into the kidney hilus. Whether these anatomical characteristics contribute to this curious pattern of incidence remains a moot point.

Failure to mention possible thymus effects in a paper dealing with TCDD exposure would be misleading. Unfortunately, data are too meager and diverse in source to assemble specific figures for presentation; however, there appears to be a reduction in thymus weight at birth in some pups from females treated with TCDD during pregnancy. In postnatal studies, there was a dose-related thymus weight decrease when compared to controls in pups weaned from mothers who received 10 or 3 μg/kg TCDD at parturition. Definitive experiments to assess thymic effect and possible impairment of cellular immunity are planned.

Discussion

The results of these studies clearly indicate that exposing pregnant mice to TCDD during the period when progeny are undergoing metanephric kidney formation or maturation leads to the development of hydronephrosis. The incidence of this defect shows a dose–response relationship as scored by the litter, mean average pup, and unilateral or bilateral kidney incidence. Association with a treated mother during the nursing period accounted for the highest incidence of hydronephrosis in the studies described. Pups probably are being exposed to TCDD by its presence in milk.

The postnatal studies subsequent to prenatal exposure to TCDD at gestation day 10 would seem on first examination to discredit the significance of kidney anomalies recorded from fetuses examined on gestation day 18. However, a comparison of the salient features of the prenatal and postnatal kidney effects reveals striking similarities: (1) the lesion in both cases is best described as a hydronephrosis; (2) there is a similar dose–response relationship of kidney effects; (3) there is a tendency for right kidney hydronephrosis at low TCDD doses. The failure to demonstrate progressive hydronephrosis postnatally in pups exposed only *in utero* is likely a function of dose and

Table 3. Hydronephrosis in C57B1/6 pups from mothers treated with TCDD at parturition.[a]

			Kidney hydronephrosis		Bilateral hydronephrosis	
TCDD dose, μg/kg	No. litters	Average no. pups/litter	No. affected litters	Mean average %	No. affected litters	Mean average %
0	4	6	0	0	0	0
10	5	6	5	75	3	40
0	4	6	0	0	0	0
3	3	5.3	3	71	0	0
0	8	8.0	0	0	0	0
1	9	7.7	5	12	0	0

[a] Pups nursing mothers treated with 10 μg/kg TCDD were reduced in size at weaning.

length of target organ exposure. This conclusion is supported by the findings that the greatest incidence of hydronephrosis occurred in those litters which were exposure to TCDD both *in utero* and during the postnatal period. It is hypothesized that the pathogenesis of the hydronephrotic syndrome due to prenatal or postnatal maternal exposure is similar. Studies which test this hypothesis are in progress.

Summary

Hydronephrotic kidneys were produced in mouse pups that nursed a mother treated with TCDD during pregnancy or at time of parturition. Variations in kidney development, consistent with hydronephrosis, were observed in fetuses examined at gestation day 18. It was hypothesized that the prenatal and postnatal kidney anomaly are of the common etiology and that the incidence and degree of hydronephrosis is a function of dose and length of target organ exposure.

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