PERINATAL TCDD EXPOSURE ALTERS SEX DIFFERENTIATION IN BOTH FEMALE AND MALE LE HOODED RATS.

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Perinatal exposure to TCDD on the 15th day of gestation (GD-15) at doses lower than or equal to 1 μg both demasculinize and feminize the Holzman (SD) male rat[1,2,3]. Mably et al. observed that TCDD-exposed male offspring had reduced anogenital distance (AGD) as neonates, reduced numbers of testicular and cauda epididymal sperm and smaller androgen-dependent sex accessory glands after puberty. In addition, they found that sexual behaviors during adulthood were altered by perinatal TCDD administration. Specifically, treated male offspring displayed longer latencies to mount and ejaculate and a greater number of mounts and intromissions were required to ejaculate. In fact, Mably et al.[1,2,3] noted permanent effects at doses about two orders of magnitude below those required to produce reproductive alterations in adult SD male rats[4,5]. Thus, their study is critical in the assessment of noncancer health effects of TCDD, because it demonstrated that the perinatal stage of life is extremely sensitive to TCDD. It was our objective to expand these observations to another strain of rat and to examine the effects of perinatal TCDD on reproductive development of female offspring as well.

In the current study, pregnant LE Hooded rats (8 per group) were dosed by gavage with 1 μg TCDD/kg in corn oil on GD 15, while controls received corn oil alone. At this time, serum testosterone (T) and in vitro T production were measured in surplus males. Maternal/pup viability and growth were monitored throughout the study, AGD was measured throughout the preweanling period, and pubertal indices were measured in males (preputial separation) and females (vaginal opening). After puberty, vaginal and behavioral (running wheel activity) estrous cycles were monitored up to 155 days of age. Some of the male offspring were necropsied at 49 days of age to compare to the numerous reproductive alterations found at this age by Mably et al[1,2,3]. Ejaculated sperm counts (ESC) were measured over three trials from 115 to 160 days of age on a biweekly basis, as permanent effects of TCDD on epididymal sperm numbers have been reported. Subsequently, the males were paired with receptive females and sexual behaviors were scored (d 163 to d 171).

Administration of 1 μg/kg of TCDD did not cause maternal death or reduce weight gain during gestation. However, TCDD did reduce pup survival during lactation by about 11 % and growth was retarded as well (Table 1). The magnitude of this effect increased throughout lactation, but after weaning, body weight differences were attenuated. AGD was also slightly reduced in TCDD-males but the effect was less than that reported for the Holzman rat (Table 1).

Table 1. Developmental effects of 1 μg TCDD/kg on GD-15 on neonatal growth and anogenital distance in male and female LE Hooded rats.

	CON-Female	TCDD-Female	CON-Male	TCDD-Male
BODY WT: DAY0	7.35	6.65 (7%)	7.41	6.94 (7%)
AGD (mm): DAY0	1.23	1.07 (13%)	2.64	2.43 (9%)
BODY WT (g): DAY3	10.7	8.95 (16%)	10.7	9.06 (8%)
AGD (mm): DAY3	1.87	1.80 (4%)	3.78	3.46 (8%)
BODY WT (g): DAY8	19.9	17.0 (15%)	20.0	17.2 (16%)
AGD (mm): DAY8	-	-	6.15	5.49 (13%)
BODY WT (g): DAY15	36.3	32.1 (11%)	38.9	27.2 (30%)
AGD (mm): DAY15	6.02	5.35 (11%)	10.4	9.16 (13%)

Values are means (% reduction due to treatment)

After puberty, a series of remarkable urogenital malformations were noted in the TCDD-treated female offspring. Minimal to severe clefting of the phallus/clitoris was noted in most of the treated females. Severe clefting was accompanied by hypospadias. In addition, vaginal opening was incomplete (many) to absent (a few) in the TCDD exposed females. Those minimally affected had a permanent "thread" of tissue across the vaginal orifice oriented in an anterior to posterior direction. In addition, the vaginal orifice appeared to be smaller than normal in most of the treated females. In contrast to the external genitalia, vaginal and behavioral (running wheel activity) estrous cyclicity were not significantly affected by TCDD treatment from puberty up to 5 months of age. It is important to caution that it is not unusual for females, treated perinatally with a hormonally active toxicant, to cycle normally for a few months and then become acyclic earlier than normal.

In the male rat, TCDD treatment delayed puberty (preputial separation) by 3 days. These results (Table 2) are similar to those reported for the Holzman rat, but the absolute differences seen in this study were of lesser magnitude. Body, ventral prostate, and seminal vesicle weights, daily sperm production and epididymal numbers were reduced by TCDD treatment. Although, testes and epididymal weights also were reduced, these differences were not statistically significant. Liver, thymus, spleen, brain, adrenal and kidney weights were unaffected by TCDD. Interestingly, in spite of the reductions in weights of the T-dependent tissues, serum T and in vitro testicular T production were normal. Although these endocrine results differ from those originally reported for the Holzman rat[1,2,3], they are consistent with more recent endocrine data from TCDD-treated Holzman rat pups[6].

Table 2. Effects of 1 μg TCDD/kg on endpoints measured in LE Hooded male rats, necropsied at 49 days of age

DOSAGE GROUP	0	1 μg
Body Weight	273 g	256
Paired Testes Wt	2.58 g	2.35
Ventral Prostate Wt	164 mg	112
Seminal Vesicle Wt	490 mg	382
Epididymal Wt	189 mg	171
Caudal Epididymal Wt	71 mg	65
Pituitary Weight	7.9 mg	8.0
Daily sperm production (TSHC/6.1)	22	15
Epididymal sperm count x 10^6	17.9	7.4
Shaded areas differ significantly from control values		

We anticipated that the observed reduction in ESCs from 4.44 x 10^8 sperm (total over three trials) in control males to 1.65 x 10^8 in the TCDD-treated males would not produce infertility, because ESCs must be reduced by more than 95% to impair fertility[7]. This is consistent with the reported reduction in cauda epididymal sperm numbers in male offspring exposed perinatally to TCDD, with no effect on fertility[3].

We observed alterations in some of the same male sex behaviors reported to be impaired in the TCDD-treated Holzman rats. For example, the total number of mounts, with and without intromission, was doubled as a consequence of perinatal

TCDD exposure and the latency to ejaculate was increased.

In conclusion, we found that perinatal exposure to TCDD at 1 μg/kg on GD-15 severely affects sex differentiation in both female and male LE Hooded rats. The females displayed urogenital malformations, while the male offspring showed functional deficits, including reduced ejaculated sperm counts, altered mating behavior and reduced accessory sex gland size. This constellation of effects on the male offspring is similar to that reported by Mably et al.[1,2,3] using Holzman SD rats. Interestingly, cleft phallus with hypospadias results from perinatal exposure to potent estrogens like DES[8]. Taken together, these data suggest that TCDD produces "estrogen-like" malformations in gestationally exposed female rats. This is not to say that TCDD is estrogenic, because TCDD did not produce many of the effects that one would expect to result from perinatal exposure to an estrogenic substance.

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