In Utero and Lactational Exposure of the Mouse to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD): Effects on Male Reproductive Tract Development

Rebecca J. Sommer[1] and Richard E. Peterson[1,2], [1]School of Pharmacy and [2]Environmental Toxicology Center, University of Wisconsin, Madison, Wisconsin, USA 53706.

Abstract

To determine whether *in utero* and lactational TCDD exposure alters male reproductive tract development and sperm production in the mouse, pregnant ICR mice were administered TCDD (1.0 or 3.0 µg/kg, po) or vehicle on gestation days (GDs) 12-14 and male offspring were evaluated from postnatal day (PND) 50-101. Perinatal TCDD exposure reduced ventral prostate weight on PND 50 and coagulating gland weight on PND 65 and 95. Body weight was reduced from PND 77-101. Testis, epididymis, vas deferens, dorsolateral prostate, and seminal vesicle weights were similar in control and TCDD-exposed male offspring. In addition, epididymal and ejaculated sperm numbers were not affected by TCDD, but the ability of male offspring to mate with untreated females appeared to be reduced in the 3.0 µg TCDD/kg treatment group . Overall, these findings demonstrate that ventral prostate and coagulating gland growth and development are the most sensitive signs of TCDD developmental male reproductive toxicity in the mouse and that this species is less sensitive than the rat and hamster to this type of toxicity.

Introduction

Male offspring of pregnant rats administered a single oral dose of 1.0 µg TCDD/kg on GD 15, have delayed separation of the prepuce from the glans penis (an androgen-mediated indication of the onset of puberty), reduced sperm numbers in the testis, epididymis and ejaculate, reduced prostate weight, and altered sexual behavior.[1,2,3,4,5] These effects occur at a maternal dose of TCDD as low as 1/100th of that needed to adversely affect the male rat reproductive system following TCDD exposure in adulthood.[6] Thus, the developing male rat reproductive system is very sensitive to TCDD exposure. The developing male reproductive system of the hamster is also sensitive to *in utero* and lactational TCDD exposure. Male offspring of pregnant hamsters administered a single oral dose of 2.0 µg TCDD/kg on GD 11 (equivalent stage of urogenital differentiation as GD 15 in the rat), have delayed preputial separation, reduced sperm numbers in the epididymis and ejaculate, sperm granuloma formation in the epididymis, and reduced seminal vesicle weights.[4] This study was conducted to determine whether the developing male mouse reproductive system is also adversely affected by TCDD. Accordingly, the effect of *in utero* and lactational TCDD exposure on preputial gland separation, reproductive organ weights, cauda epididymal sperm number and ejaculated sperm number were determined in the mouse.

Methods

Timed pregnant ICR mice (Harlan Sprague-Dawley, Inc., Madison, WI) arrived on GD 7 (GD 0 = sperm positive) and were housed individually in plastic cages (28 x 18 x 12 cm) containing aspen chip bedding. Mice were maintained at 22 ± 1°C, 35 ± 5% humidity, with a 12 hr:12 hr photoperiod with lights on at 0600 hr and off at 1800 hr. Feed (Formulab Richmond Standard Diet, PMI Feeds, St. Louis, MO) and tap water were provided *ad libitum*. Dams were administered an oral dose of TCDD (1.0 or 3.0 μg/kg, 98% purity, Cambridge Isotope Laboratories, Woburn, MA) or an equal volume of vehicle (2 ml/kg, 95% corn oil/5% acetone) for three consecutive days, GDs 12-14.

The mouse reproductive tract completes development one to three days earlier than the rat (Table 1). Thus, to assure that TCDD exposure occurs during these critical stages of mouse reproductive tract development, TCDD was administered daily on GDs 12, 13 and 14. Since the threshold for cleft palate formation in the mouse is 3 μg TCDD/kg administered on GDs 10-13[7] and 6 μg TCDD/kg on GD 12 results in 6% cleft palate formation[8], a small percentage of the TCDD exposed mice in the present study were expected to develop cleft palate.

Table 1. Comparison of mouse and rat reproductive tract development (gestation days).

Species	Implantation	Testicular Differentiation	Androgen Biosynthesis	Mullerian Duct Regression	Wolffian Duct Regression
Mouse	4.5	12	13	14	15
Rat	5.5	14	15	17	18

Adapted from Reference 9

On the day of birth (PND 0), number of live offspring were recorded, and litters were adjusted to 4 males and 4 females. Litter independence was maintained. On PND 21, offspring were weaned and housed with same-sex litter mates.

On the day of termination (PND 50, 65, or 95), testes, epididymides, vas deferens, ventral prostate, dorsolateral prostate, seminal vesicles, and coagulating glands were removed, trimmed of fat, and weighed. On PND 65, the left cauda epididymis was macerated and homogenized in 15 ml of 0.9% saline containing 0.05% Triton X-100 and 0.01% thimerosal, for 2 min, at low speed, in a semimicro Waring blender. Cauda epididymis homogenates were diluted to approximately 1 x 10^6 sperm/ml and counts from 4 hemacytometer chambers were made in duplicate and averaged. Male reproductive organ weights and cauda epididymal sperm number were determined for 1 male/litter from 6-7 different litters/treatment.

Ejaculated sperm number was determined from 2-4 mating trials conducted at one-week intervals from PND 70-101 . Each male was paired with one untreated, sexually receptive (in estrus), adult female, overnight, in the male's home cage. On the following morning, females were euthanized and their uterine contents flushed into 5 ml of 0.9% saline containing 0.05% Triton X-100 and 0.01% thimerosal. Ejaculated sperm number for each male was determined as the mean ejaculated sperm number of 2-4 mating trials. Treatment group means were based on 1 male/litter from 3-5 different litters/treatment. Mice used to determine ejaculated sperm number were not used in reproductive organ weight or cauda epididymal sperm number determinations.

Statistical analysis was performed using Statistica (StatSoft, Tulsa, OK). All data were analyzed by one-way analysis of variance using Fisher's LSD as the multiple comparison test. Levene's test was used to assess homogeneity of variance. Significance was set at $p < 0.05$.

Results and Discussion

TCDD treatment caused no overt toxicity to the dams as assessed by body weight and visual inspection. Offspring mortality (based on implantation sites) was not significantly increased by TCDD exposure, even though 2 of 9 and 2 of 8 litters in the 1.0 and 3.0 μg TCDD/kg treatment groups, respectively, had at least 1 pup die by PND 2 with cleft palate. *In utero* and lactational TCDD exposure did not alter anogenital distance and crown rump length (measured on PND 3), age at preputial separation, or weekly body weights from PND 1-70. However, from PND 77-101, TCDD significantly decreased body weights to 93% and 91% of control in the 1.0 and 3.0 μg TCDD/kg treatment groups, respectively.

Testis, epididymis, vas deferens, dorsolateral prostate, and seminal vesicle weights were not affected by TCDD. In contrast, ventral prostate weight was decreased at PND 50 but not at later times, whereas weight of the coagulating glands was decreased on PNDs 65 and 95 but not on PND 50 (Fig. 1). Ventral prostate and coagulating gland weights remained significantly decreased when expressed as relative organ weights (organ weight/body weight; results not shown).

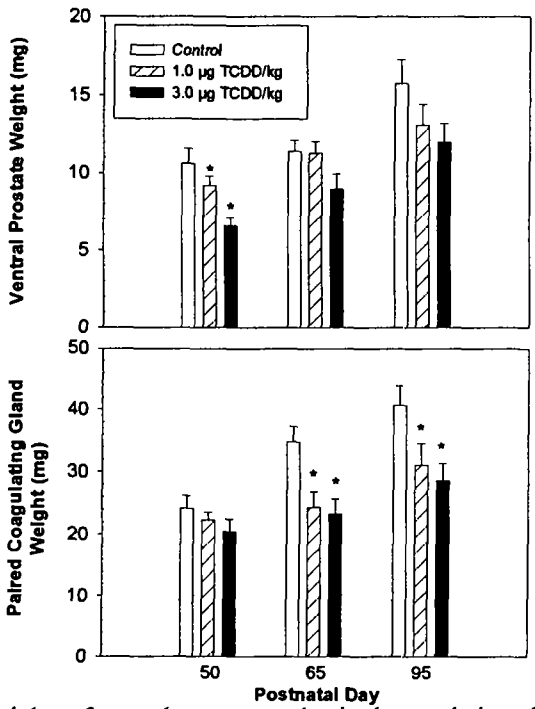

Figure 1. Absolute weights of ventral prostate and paired coagulating glands on PNDs 50, 65 and 95 of male mouse offspring of dams exposed to 0, 1.0, or 3.0 μg TCDD/kg dam body weight administered on GD 12-14. *Significantly different from control, $p<0.05$.

A similar pattern of decreased prostate weight early in development and decreased coagulating gland weight later in adulthood has been observed in the rat following *in utero* and lactational exposure to TCDD.[5] However, in the rat both ventral and dorsolateral prostate weights were decreased by perinatal TCDD exposure, whereas in the mouse there was no effect on dorsolateral prostate weight.

TCDD exposure did not decrease either cauda epididymal or ejaculated sperm numbers. However, the percentage of successful matings between untreated, sexually receptive females and control or TCDD-exposed male offspring ([number of pairings resulting in sperm recovery in the female uterus/number of pairings] X 100) was variable. Males in the control, 1.0, and 3.0 μg TCDD/kg treatment groups successfully mated in 61%, 50%, and 29% of the pairings, respectively. All mice in the control and 1.0 μg TCDD/kg treatment groups mated at least once in 3-4 pairings, whereas 4 of 7 mice in the 3.0 μg TCDD/kg treatment group did not have a single successful mating in 4 pairings. The decrease in successful matings may have resulted from overt toxicity, as body weights of the TCDD-exposed mice were less than control on PND 77-101. Another possibility is that *in utero* and lactational TCDD exposure which partially demasculinizes sexual behavior in the rat, also does so in the mouse.

Recently, Theobald and Peterson conducted an *in utero* and lactational TCDD dose-response study to determine whether higher maternal doses of TCDD than used in the present study would affect male mouse reproductive system development.[10] Pregnant ICR mice were administered a single oral dose of 0, 15, 30, or 60 μg TCDD/kg dam body weight on GD 14. Consistent with the present study, the most sensitive endpoints in male offspring were decreased ventral prostate and coagulating gland weights. Unlike the transitory decrease in ventral prostate weight observed at lower doses in the present study, the decrease in ventral prostate weight induced by the higher doses of TCDD persisted from PND 44-128. The treatment groups 30 and 60 μg TCDD/kg also had decreased epididymal sperm numbers on PND 65, but not on PND 114-128.[10] In contrast, *in utero* and lactational exposure to 1.0 μg TCDD/kg dam body weight, administered on GD 15, permanently decreases epididymal sperm number in the rat.[4] Taken together, these findings demonstrate that the mouse is less sensitive to TCDD-induced developmental reproductive system toxicity than either the rat or hamster.

Acknowledgments

Supported by NIH grant ESO1332.

Literature Cited

(1) Mably, T.A.; Bjerke, D.L.; Moore, R.W.; Gendron-Fitzpatrick, A.; Peterson, R.E. *Toxicol. Appl. Pharmacol.* **1992a**, 114, 118-126.
(2) Mably, T.A.; Moore, R.W.; Goy, R.W.; Peterson, R.E. *Toxicol. Appl. Pharmacol.* **1992b**, 114, 108-117.
(3) Mably, T.A.; Moore, R.W.; Peterson, R.E. *Toxicol. Appl. Pharmacol.* **1992c**, 114, 97-108.
(4) Gray, L.E., Jr.; Kelce, W.R.; Monosson, E.; Ostby, J.S.; Birnbaum, L.S. *Toxicol. Appl. Pharmacol.* **1995**, 131, 108-118.
(5) Roman, B.L.; Sommer, R.J.; Shinomiya, K.; Peterson, R.E. *Toxicol. Appl. Pharmacol.* **1995**, 134, 241-250.
(6) Peterson, R.E.; Theobald, H.M.; Kimmel, G.L. *Crit. Rev. Toxicol.* **1993**, 23, 283-335.
(7) Birnbaum, L.S.; Harris, M.W.; Miller, C.P.; Pratt, R.M.; Lamb, J.C. *Teratol.* **1986**, 33, 29-35.
(8) Abbott, B.D.; Birnbaum, L.S. *Toxicol. Appl. Pharmacol* **1989**, 99, 276-286.
(9) Greco, T.L.; Duello, T.M.; Gorski, J. *Endocrine Reviews* **1993**, 14, 59-71.
(10) Theobald, H.M.; Peterson, R.E. *Toxicol. Appl. Pharmacol.* **1997**, in press.