# *IN UTERO* AND LACTATIONAL TCDD EXPOSURE IN THE MOUSE: IMPAIRED PROSTATE DEVELOPMENT AND FUNCTION

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## Introduction

Previous studies in rats have identified impaired growth and development of the prostate as a sensitive endpoint of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity[1]. The prostate develops from the fetal urogenital sinus (UGS). On gestation day (GD) 16 in the mouse, prostate development is characterized by the formation of solid buds of basal epithelial cells that invade the mesenchyme. After birth, branching morphogenesis and canalization of the prostatic ducts occurs and the basal epithelial cells differentiate into luminal epithelial cells that serve a ductal secretory function[2]. *In utero* and lactational TCDD exposure impairs growth and development of the mouse prostate in a lobe-specific manner. The ventral prostate (VP) is most sensitive to disrupted development and function by TCDD, followed by the anterior prostate (AP), and dorsolateral prostate (DLP). Using AhR knockout mice it was found that the disruptive effects of TCDD on prostate development are AhR-dependent[3].

Results of cross-fostering experiments in the mouse have established the role of prenatal versus postnatal TCDD exposure on the impairment of prostate growth. It was found that TCDD acts both prenatally and postnatally to inhibit prostate growth. However, prenatal exposure is far more disruptive[4,5]. Also *in utero* exposure to TCDD decreased the formation of prostatic buds from the UGS epithelium. More specifically, scanning electron microscopy (SEM) of the mouse UGS epithelium on GD 18 (after removal of the mesenchyme by trypsin digestion) revealed agenesis of ventral buds (VB) and decreased numbers of dorsolateral buds (DB) in fetuses exposed to TCDD beginning on GD 13[1].

The objective of the present study was to determine if TCDD exposure beginning on GD 13 alters the time course of UGS epithelial development and prostatic bud formation. An additional objective was to determine in a cross-fostering study the contribution of *in utero* versus lactational TCDD exposure on the maturation of secretory function of the different prostate lobes based on mRNA abundance of lobe-specific secretory proteins on postnatal day (PND) 35.

## Materials and Methods

Timed pregnant C57Bl/6J mice were dosed with 5 μg/kg TCDD or vehicle (95% corn oil, 5% acetone) on GD 13. Male fetuses were sacrificed and the UGS complex was collected daily from GD 14 until the day of birth designated as PND 0. Limited trypsin digestion of UGS tissue was used to separate the uroepithelial layer from the surrounding mesenchyme. The isolated UGS epithelium with associated prostatic buds intact was fixed in 2.5% glutaraldehyde and subsequently evaluated by SEM for UGS epithelial development and prostatic bud formation.

A cross-fostering study was also conducted. Timed pregnant C57Bl/6J dams were dosed with 5 μg/kg TCDD or vehicle on either GD 13. Upon delivery litters were fostered to dams of the same or opposite treatment. Four treatment groups were assessed: (1) male offspring not exposed to TCDD by either route (Control), (2) male offspring only exposed to TCDD *in utero* beginning on GD 13 (IU 13), (3) male offspring only exposed to TCDD via lactation from dams that were dosed with TCDD on GD 13 (L13), or (4) male offspring exposed both *in utero* and via lactation to TCDD from dams dosed with TCDD on GD 13 (IUL 13). In addition, a fifth group of male offspring that was exposed *in utero* and by lactation to the same dose of TCDD but beginning on GD 16 (IUL 16) was also used. All pups were weaned on PND 21. On PND 35 juvenile male offspring were necropsied and the VP, DLP, and AP were dissected and weighed. To evaluate prostate secretory function, samples of individual lobes were snap frozen in liquid nitrogen for mRNA quantification. LightCycler RT-PCR was used to determine mRNA abundance of the following prostate lobe-specific, androgen-dependent, secretory proteins: MP25 (VP), renin-1 (AP) and probasin (DLP). All LightCycler results were normalized against cyclophilin mRNA abundance.

**Results and Discussion**

Time Course Study. *In utero* exposure to TCDD delayed development of the UGS epithelium and impaired prostatic bud formation in a region-specific manner (Figure 1). The time course of UGS development and prostatic bud formation in representative control male mouse fetuses (top panel) and representative TCDD-exposed male mouse fetuses (bottom panel) is shown.

In the control fetus anterior prostatic buds (AB) form near the point where the Wolffian duct meets the UGS. Dorsal buds (DB) emerge from the dorsal region of both the bladder neck (BN) and UGS while lateral buds (LB) are located on the lateral region of the UGS. Ventral buds (VB) rise from the ventral region of the BN. These buds develop into the different prostate lobes (AB into AP, DB and LB into DLP, and VB into VP). In the control fetus there is no evidence of either BN development or prostatic buds emerging from UGS epithelium on GD 14 (data not shown). AB and BN first become evident on GD 15. Extension of the BN begins in the control fetus on GD 16 and is complete by the end of GD 18. DB formation begins on GD 16 and continues to GD 18. Formation of LB and VB starts on GD 17 and are complete on GD 18. On PND 0 no additional buds are observed in the newborn pup, although, existing buds are larger in size (data not shown).

TCDD exposure disrupted development of the UGS epithelium and prostatic bud formation. AB formation, BN extension, DB formation and LB formation were all delayed about 24 hr by 5 μg/kg of TCDD administered to the dam on GD 13. On the other hand, VB were absent from the UGS on PND 0, which is two days after they should have been formed. This greater impairment of VB formation is consistent with the VP being more severely hypogenic on PND 90 than the other prostate lobes[6].

Cross Fostering Study. The results of this study demonstrated that disruption of prostatic bud formation caused by TCDD exposure had a long-term impact on the maturation of prostate secretory function. The effect was greatest for the VP followed by AP and DLP as described below. The results are presented in Table 1.

Ventral prostate. VP relative weight and MP25 mRNA abundance on PND 35 were most severely reduced after IUL 13 and IU 13 TCDD exposure (Table 1). A less dramatic decrease was seen

after IUL 16 TCDD exposure. These findings suggest that GD 13-16 is a critical period for *in utero* TCDD exposure to disrupt VP growth and secretory function. L13 TCDD exposure caused significant, yet, smaller decreases of both end points.

Dorsolateral prostate. DLP relative weight and probasin mRNA abundance, were similarly decreased in IU 13 and L 13 TCDD exposures. In addition, there was no preference between IUL 13 and IUL 16 exposure. The absence of a prenatal critical period for TCDD effects on the DLP may be due to the larger number of DBs and LBs and possibly multiple waves of bud formation.

Anterior prostate. The most severe reduction in relative weight of the AP was seen after IUL 13 TCDD exposure, followed by IU 13 exposure. AP growth was least affected after L 13 TCDD exposure. Like VP, AP growth and development was most sensitive to TCDD exposure between GD 13 and GD 16. However, There was no critical period for TCDD effects on the development of AP secretory function, because renin-1 mRNA abundance was similarly depleted regardless of any combination of perinatal TCDD exposure.

*In utero* and lactational exposure to TCDD may impair mouse prostate secretory function on PND 35 by decreasing the population of androgen responsive luminal epithelial cells in the ducts[7], decreasing androgen-responsiveness of these secretory epithelial cells[8], or both. The mRNA quantification of cytokeratin 8, the mature epithelial cell specific marker, is in progress. When combined with mRNA abundance data for the secretory proteins, we can better understand the response of these three prostate lobes to TCDD.

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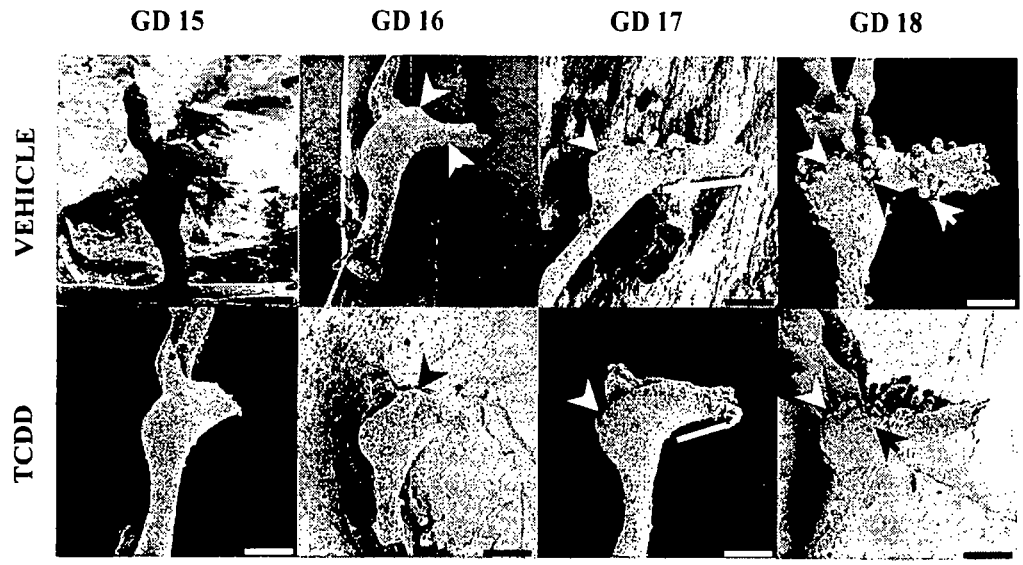


**Figure 1.** *In utero* and lactational TCDD exposure delays urogenital complex development and decreases prostatic bud formation in the male mouse fetus. The UGS of vehicle and TCDD exposed fetuses was processed for SEM. Representative SEM photomicrographs are shown. AB: anterior buds, BN: bladder neck, DB: dorsal buds, LB: lateral buds and VB: ventral buds.

**Table 1.** *In utero* versus lactational TCDD exposure: Differential effects on relative prostate lobe weight and mRNA abundance of prostate lobe-specific secretory proteins.

| | Ventral Prostate | | Dorsolateral Prostate | | Anterior Prostate | |
|---|---|---|---|---|---|---|
| Treatment group[1] | Relative weight (mg/g body) | mRNA abundance (MP25/Cy)[2] | Relative weight (mg/g body) | mRNA abundance (Probasin/Cy)[3] | Relative weight (mg/g body) | mRNA abundance (Renin-1/Cy)[4] |
| Control | 0.24 ± 0.01 | 1400 ± 140 | 0.32 ± 0.01 | 0.06 ± 0.01 | 0.58 ± 0.02 | 2.50 ± 0.40 |
| | Relative weight (% of control) | MP25 mRNA (% of control) | Relative weight (% of control) | Probasin mRNA (% of control) | Relative weight (% of control) | Renin-1 mRNA (% of control) |
| Control | 100 ± 4 | 100 ± 10 | 100 ± 3 | 100 ± 14 | 100 ± 6 | 100 ± 16 |
| IUL 13 | 14 ± 4 *[5] | 3 ± 1 * | 47 ± 3 * | 15 ± 3 * | 33 ± 4 * | 28 ± 6 * |
| IU 13 | 9 ± 2 * | 3 ± 1 * | 74 ± 8 * | 51 ± 4 * | 51 ± 7 * | 24 ± 6 * |
| L 13 | 59 ± 5 * | 42 ± 10 * | 80 ± 4 * | 40 ± 4 * | 78 ± 8 * | 32 ± 5 * |
| IUL 16 | 31 ± 4 * | 11 ± 2 * | 49 ± 6 * | 15 ± 12 * | 50 ± 6 * | 25 ± 7 * |

[1] Control =*in utero* and lactational vehicle exposure from GD 13; IUL 13 = *in utero* and lactational TCDD exposure from GD 13; IU 13 = *in utero* TCDD exposure from GD 13; L 13 = lactational TCDD exposure from dam dosed on GD 13; IUL 16 = *in utero* and lactational TCDD exposure from GD 16.
[2] MP25/Cy = cyclophilin normalized MP25 mRNA abundance.
[3] Probasin/Cy = cyclophilin normalized probasin mRNA abundance.
[4] Renin-1/Cy = cyclophilin normalized renin-1 mRNA abundance.
[5] Significance level $p < 0.05$ compared to vehicle control.