EFFECTS OF *IN UTERO* AND LACTATIONAL TCDD EXPOSURE ON MALE REPRODUCTION PATTERN IN THREE DIFFERENTIALLY TCDD SENSITIVE RAT LINES

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Introduction

The endocrine system presents a number of target sites for the induction of adverse effects by ubiquitous environmental contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In previous studies, various dose-dependent effects on male offspring androgenic status were seen, when TCDD was administered to pregnant female rats. These low-dose effects are essential in terms of dioxin risk assessment. However, the sensitivity of even the same species to male reproduction defects has varied in different studies. Mably *et al.* reported a significant reduction in daily sperm production and epididymal sperm reserves in Holzman rat male offspring after maternal dose of 0.064 μg/kg[1]. Anogenital distance on postnatal day (PND) 1 and 4, and the size of sex-accessory glands (ventral prostate, seminal vesicles, testes) were significantly reduced. Yet, no effect on fertility was seen. On the other hand, in a similarly designed study, Gray *et al.* found transient reductions in ventral prostate and seminal vesicle weights and permanent reduction in epididymal sperm reserves in Long-Evans Hooded rats after a maternal dose of 0.8 μg TCDD/kg[2]. Testis or daily sperm production were not affected. Furthermore, Ohsako *et al.* detected a significant reduction in size of ventral prostate and reduced anogenital distance (PND 1 and 4) in Holzman rats only at the highest maternal dose (0.8 μg/kg)[3]. Daily sperm production, epididymal sperm reserves, epididymal weight or testis weight were not affected.

Adult Han/Wistar (Kuopio; H/W) rats are extremely resistant and Long-Evans (Turku; L-E) rats exceptionally susceptible to TCDD in terms of acute lethality. H/W type aryl hydrocarbon receptor (AHR) gene has been shown to harbor a point mutation, which results in abnormal C-terminus transactivation domain.[4] This change is assumed to result in altered activation of genes related to lethality. The H/W-type "TCDD resistance genes", the altered *Ahr* and another unknown gene were segregated to new rat lines, designated A, B and C[5]. Line A has the resistant *Ahr* allele (Ahr^{hw}), line B the other, currently unknown resistance gene of H/W rat (B^{hw}). Line C rats have neither of these H/W-type resistance genes. Lines A, B, and C exhibit a highly different LD_{50} values for TCDD: >10 000, 830, and 40 μg/kg in males, respectively.

This study was conducted to determine whether the sensitivity difference shown for lethality between lines A, B and C is also present in defects of developing male rat reproductive system, and if the sensitivity to these defects is dependent on the resistance genes.

Methods

Line A, B and C rats were bred in the SPF-barrier unit of the National Public Health Institute (Kuopio, Finland). Adult (12-15-week-old) female rats in estrus were mated. Pregnant A, B and C line dams were administered a single oral dose of 0.03-1 μg TCDD/kg (in corn oil) on gestational day (GD) 15 (GD 0 = sperm positive). Control animals received corn oil. There were 5-8 pregnant dams at each dose level. One day after birth (PND 1), offspring number was recorded and litters were adjusted to 3 males and 3 females, when possible. On PND 28, offspring were weaned and housed with same-sex littermates.

On the day of termination (PND 70), testes, right epididymis, ventral prostate, seminal vesicles and thymus were removed, trimmed, and weighed. Cauda epididymis was weighed separately. Right cauda epididymis and testis were frozen for further sperm counts. For daily sperm production and epididymal sperm count the frozen testes and cauda epididymis were homogenized in 0.9% saline containing 0.05% Triton X-100 and 0.01% thimerosal. Homogenates were diluted to approximately 1 x 10^6 sperm/ml and counted using hemocytometer.

Results and Discussion

TCDD treatment caused no overt toxicity to the dams as assessed by the body weight and visual inspection. There were only slight effects on measured endpoints in A, B and C rat strains. Maternal dose of 1 μg TCDD/kg marginally, but significantly decreased anogenital distance in line B and C, but had no effects to line A male offspring (PND 1). When anogenital distance was normalized to the crown-rump length, the decrease was significant only in line C rats (PND1). In lines B and C body weights were slightly decreased in the 1 μg TCDD/kg treatment group (PND 1-7). After PND 7 and until the day of termination PND 70 the decrease was significant only in line C offspring (1 μg TCDD/kg). Cauda epididymis and ventral prostate weights (Fig. 1 left) were significantly ($p<0.05$) affected by the maternal dose 1 μg TCDD/kg only in lines B and C. After normalizing to the body weight, the decrease remained significant only in line B rats relative ventral prostate weights ($p<0.01$). Daily sperm production (Fig. 1 right) was significantly ($p<0.01$-0.05) decreased in line B rats at the maternal dose of 1 μg TCDD/kg and in line C rats at 0.1 μg TCDD/kg. Also epididymal sperm reserves were significantly ($p<0.01$) decreased in line B and C rats at 1 and 0.3 μg TCDD/kg, respectively.

Lines A, B and C showed only slight sensitivity differences after *in utero* and lactational TCDD exposure as assessed on PND 70. The results demonstrate that the resistance genes Ahr^{hw} and B^{hw} do not profoundly affect the sensitivity of male reproduction parameters to *in utero* and lactational TCDD exposure. However, line A rats seem to be somewhat more resistant to the effects on male reproductive toxicity at the studied dose levels. In addition, adult H/W rats (having both resistance genes Ahr^{hw} and B^{hw}) seem to be exceptionally resistant to the testicular toxicity of TCDD at dose levels up to 1000 μg/kg[6]. Previous studies suggest that PND70 may be too late timepoint to detect effects on ventral prostate weights, but the effects on epididymal sperm reserves should be permanent[2]. In the study sperm production and reserves proved to be susceptible to the *in utero* and lactational TCDD exposure. For organ weights the only significant effects were seen at the highest maternal dose of 1 μg TCDD/kg. These results suggest that at low dose levels the sensitivity to TCDD-induced male reproductive toxicity during development is only slightly strain dependent.

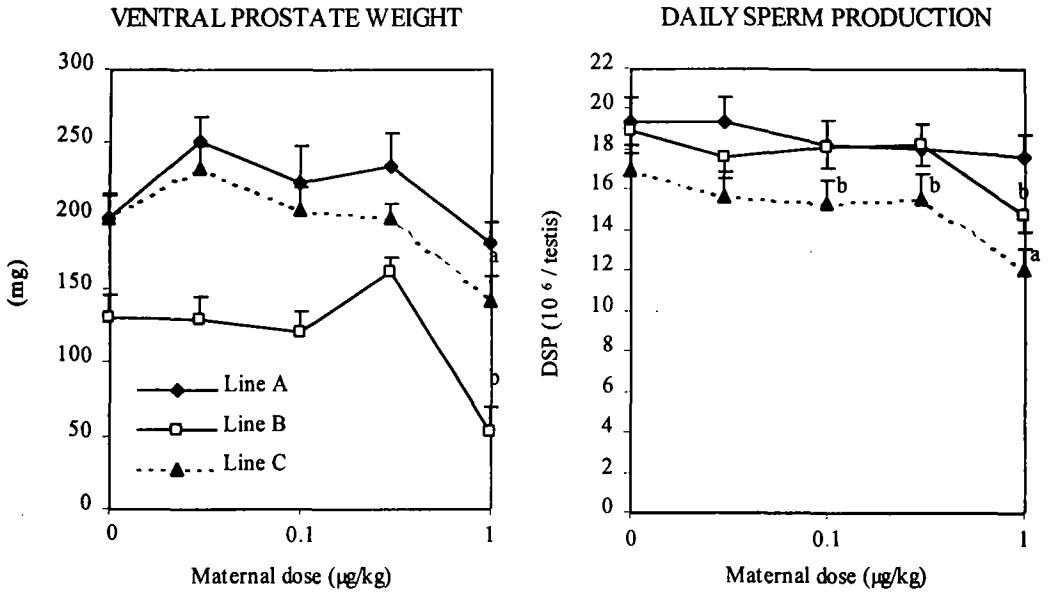

Figure 1: Effects of a single maternal dose of TCDD on the ventral prostate weight (left) and the daily sperm production (right) (PND70). Group average ± SE, n=10-16. Significance: a ($p<0.01$) and b ($p<0.05$) compared with the dose group of 0 µg/kg.

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