2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) Alters Epithelial Development of the Seminal Vesicles

Jonathan T. Hamm [1,2], Barney R. Sparrow [3], Douglas Wolf [2] and Linda S. Birnbaum [2].

[1] Curriculum in Toxicology, University of North Carolina, Chapel Hill, North Carolina 27599-7270; [2] National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711; [3] National Research Council-U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711.

Introduction

In utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin has been shown to perturb the development of the male sex accessory glands in the rat[1,2]. Roman *et al.*[3] reported that *in utero* and lactational exposure to TCDD resulted in alterations in the budding of the fetal prostate as well as postnatal changes in epithelial differentiation and smooth muscle thickness. Observations made on the seminal vesicles demonstrate delayed growth of this tissue in treated animals during the first few postnatal months. In the Holtzman rat, this delay is apparent by postnatal day 32 when androgens have been shown to peak within the seminal vesicles[4]. In addition, this postnatal date corresponds to a period of rapid proliferation and differentiation within the seminal vesicle epithelium[5] .

Analysis of seminal vesicles over time suggests that the changes in weight may be transient[1,6] . However, Gray *et al.*[2] reported that gestational exposure to 1.0 ug/kg of TCDD caused a significant decrease in seminal vesicle weights of 8-11 month old offspring. Therefore, we wanted to expand the time course for changes in seminal vesicle weights in the Long Evans rat and exam the histology of the tissue at time points exhibiting differences in the weight of the organ.

Materials and Methods

Chemicals. TCDD (>98% purity) in acetone (1 mg/10 ml) was obtained from Radian Corp.(Austin, TX). For the preparation of dosing solution a volume of stock TCDD was added to corn oil and the acetone removed by evaporation using a Savant SpeedVac (Savant Instruments Inc., Farmingdale, NY). Following evaporation, additional corn oil was added to the achieve the desired TCDD concentration. All other chemicals were from commercial sources and were of the highest purity available.

Animals. Time-pregnant Long Evans rats (gestational day 9/ day after mating= GD0) were obtained from Charles River Breeding Laboratories (Raleigh, NC). Females were housed in plastic cages containing heat-treated pine shavings (Beta Chips, North Eastern Products Inc., Warrensburg, NY) and given food (Purina 5001 Rodent Chow, Ralston Purina Co., St. Louis, MO) and water *ad libitum*.Dams were treated by oral gavage on gestational day 15 with 1.0ug/kg in corn oil or corn oil only for controls in a dosing volume of 5ml/kg. Litters were culled to 5 males and 3 females on postnatal day 4. At weaning, animals were housed as above in unisexual groups of 2 to 3 rats/cage. Male pups (N=10), 1-2 per litter, were sacrificed on PNDs 10, 25, 32, 49, 63 or 120 and organ weights taken. In addition, seminal vesicles were fixed in 10% neutral-buffered formalin for histological examination.

Histology. Seminal vesicles from PND32 animals were paraffin processed and sections stained with hematoxylin and eosin or immunohistochemical staining for PCNA or vimentin using kits supplied by Santa Cruz Biotechnology (Santa Cruz, CA) and used according to the manufacturers instructions and counterstained with hematoxylin. In addition, sections were stained with trichrome stain in order to identify collagen.

RT-PCR Analysis of Androgen Receptor Expression. Seminal vesicles from PND25 rats were removed, snap frozen in liquid nitrogen and stored at -80°C. Tissues were removed from the freezer and RNA isolated individually using Trizol reagent (GibcoBRL, Grand Island, NY) followed by reextraction with acid phenol:chloroform. The RNA samples were checked for purity and quantified using A260/280 ratios. Expression of androgen receptor was quantified using competitive RT-PCR with primers and internal competitor RNA developed in this laboratory.

Results

In utero and lactational exposure to TCDD resulted in decreases in seminal vesicle weight similar to those observed in the Holtzman rat[4] ; while TCDD did not affect the weight of the seminal vesicles by PND25, PND32 tissue was significantly smaller ($p<0.05$).

Histological analysis of PND32 SV revealed a lower degree of epithelial branching in treated pups. At higher magnification, changes in epithelial cell morphology were apparent. While the epithelium of control animals was characterized by folded columnar cells, the epithelium from treated animals showed a lower degree of folding and rounded cells predominated. In addition, it was noted that the layer surrounding the epithelium of the seminal vesicles appeared thicker in treated pups. This layer subsequently stained positively for collagen using trichrome staining.

Analysis of androgen receptor mRNA expression failed to reveal a difference between control and treated PND25 seminal vesicles. Others have shown that the levels of androgens within the seminal vesicle at PNDs 25, 32 and 49[4] as well as the levels of androgen receptor protein at later postnatal points[2] is not affected by this exposure regime.

We are currently examining the histology of seminal vesicles from PND10 through 63 for changes in epithelial growth and differentiation. These same tissues will also be examined for changes in the extracellular matrix.

Conclusions

In utero and lactational exposure to TCDD alters the postnatal growth and differentiation of seminal vesicle epithelium. Interestingly, others have reported retarded epithelia development of the prostate[3] and mammary gland[7] following exposure to TCDD. This conserved pattern of effects on the epithelium should be useful in elucidating the mechanism of TCDD-induced reproductive alterations.

TABLE 1
SEMINAL VESICLE WEIGHTS IN CONTROL AND 1.0 ug/KG TCDD TREATED PUPS

CONTROL				
PND	**BODY**	**SEMINAL VESICLE TISSUE**	**SEMINAL VESICLE FLUID**	**TOTAL SV**
15	31.2±1.2			10.3±1.5
25	74.8±1.9			16.1±1.8
32	122.6±2.5			59.6±11.1
49	279.0±5.9	323.5±74.1	141.5±53.9	465.0±113.0
63	346.0±6.6	530.8±89.2	344.6±68.1	875.4±136.7
120	678.0±17.0	991.7±60.1	650.7±35.2	1642.4±78.8
	TCDD			
15	31.2±0.8			9.8±0.9
25	69.2±3.4			16.9±1.7
32	117.2±2.6			36.7±9.3*¤
49	257.0±10.7	223.7±57.7*¤	80.5±44.8*¤	304.1±66.4*¤
63	316±6.8	398.9±47.4*¤	312.7±67.1	711.6±77.7*¤
120	563±13.9	744.2±35.4*¤	564.4±36.9	1308.5±63.2*

Body weight reported in grams; all other tissue weights in milligrams. Seminal vesicle tissue is the weight of the paired seminal vesicles and the attached coagulating glands after expression of the fluid contents. Total SV is the combined weight of the seminal vesicles and there fluid contents. * Represents a significant difference ($p<0.05$) from control in the tissue weight. ¤ Represents a significant difference ($p<0.05$) from control in the tissue to body weight ratio.

Acknowledgments
The authors of this poster want to thank Vicki Richardson, David Ross and Dr. Brian Slezak for their help with necropsies. Financial support for this work was provided by the U.S. Environmental Protection Agency Cooperative Training Agreement (#CT902908) with the University of North Carolina, Chapel Hill, NC 27599-7270. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. (This abstract does not necessarily represent USEPA policy.)

References

1. Mably T.A., Moore, R.W. and Peterson, R.E. *Toxicol. Appl. Pharmacol.* **1992,** 114, 97-107.
2. Gray, L.E., Kelce, W.R., Monosson, E., Ostby, J.S. and Birnbaum, L.S. *Toxicol. Appl. Pharmacol.* **1995, 131**, 108-118.
3. Roman, B.L., Timms, B.G., Prins, G.S. and Peterson, R.E. *Toxicol. Appl. Pharmacol.* **1998,** 150, 254-270.
4. Roman, B.L., Sommer, R.J., Shinomiya, K. and Peterson, R.E. *Toxicol. Appl. Pharmacol.* **1995,** 134, 241-250.
5. Fawell, S.E. and Higgins, S.J. *Mol. Cell. Endocrinol.* **1986,** 48, 39-49.
6. Gray, L.E., Ostby, J.S. and Kelce, W.R. *Toxicol. Appl. Pharmacol.* **1997,** 146, 11-20.
7. Furth, P.A., Lewis, B., Lewis, A., Sommer, R., Peterson, R.E. and Flaws, J. Toxicologist **1999**, 48: 1-S(#1008).