POSTNATAL DISPOSITION OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN IN THE LONG EVANS RAT FOLLOWING GESTATIONAL EXPOSURE

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Introduction

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) causes a wide range of adverse effects including alterations in reproductive development[1]. *In utero* and lactational exposure to TCDD has been shown to perturb the development of the male sex accessory glands in the rat[2,3]. Roman *et al.*[4] reported that *in utero* and lactational exposure to TCDD resulted in alterations in the budding of the fetal prostate. Hurst et. al. [5] examined the disposition of TCDD within embryo/fetal and maternal tissues and developed the association between tissue concentrations and the incidence of certain reproductive-developmental alterations[6].

While sufficient data exist to suggest prenatal alterations underlie the effects seen in the prostate and vaginal canal, the critical window for effects on other tissues including the seminal vesicles remains unclear. However, dosing as late as postnatal day 1 causes significant decreases in seminal vesicle growth and development (unpublished observations this laboratory). Therefore, it was of interest to determine postnatal disposition of TCDD within offspring exposed through *in utero* and lactational exposure.

Materials and Methods

Chemicals. [3H]TCDD (decay corrected specific activity of 24.05 uCi/nmol TCDD) was obtained from Chemsyn Science Laboratories (Lenexa, Kansas). Dosing solution was prepared by adding [3H]TCDD in toluene to corn oil and removing solvent by evaporation using a Savant Speed-Vac (Savant Instruments Inc., Farmingdale, NY).

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.*

Treatment and Tissues. Pregnant dams (N=34) were dosed by oral gavage with 0.78ug TCDD/kg/5mls corn oil on GD15. Litters were standardized to 5 males and 3 females on postnatal day 4. Dams (N=5) were euthanized on postnatal days 1, 4, 7, 15 and 25 and liver, blood, adipose and skin taken for analysis. On PNDs 1, 4, 7, 15, 25, 32, 49,63 and 129, 1 male and 1 female pup

from each of 5 litters were euthanized and necropsied. Tissues collected from pups included blood, liver, kidney, spleen, thymus, lung, brain, muscle, skin, adipose, stomach contents, and intestines/intestinal contents. On or before PND15, the intestine and contents were sampled, whereas after PND15 the intestine was subdivided into large and small and the contents removed for sampling. Reproductive tract tissues included seminal vesicle, prostate, testis and epididymis in the male and ovary and uterus in the female. At earlier timepoints, tissues from several pups within a litter were pooled to give an adequate sample size. Finally, whole pups were collected on PNDs 1, 4, 7, 15, 25 and 32 for determination of total body burdens.

Oxidation and quantitation of samples. Tissues were oxidized using a Packard 307 Sample Oxidizer with an Oximate 80 Robotic Operator (Packard, Downers Grove, IL) and samples counted on a Beckman Model LS6000 LL liquid scintillation counter using Monophase S.

Statistical analysis. Data was evaluated for statistical significance using StatView 4.5 (Abacus Concepts, Inc., Berkeley, CA). A one-way analyses of variance (ANOVA) followed by Fisher's PLSD test as a post hoc test was performed and a level of $p<0.05$ defined statistically significant difference.

Results and Discussion

On PND1, the maternal liver to fat ratio was 2 and liver had the highest TCDD concentration. Following PND1, TCDD concentrations rapidly decreased in all maternal tissues. In contrast, the concentration in adipose remained steady until PND4 and decreased slowly thereafter so that the liver to fat ratio fell below 1.

In the pups, whole body tissue concentrations peaked at around PND4, decreased slowly until PND15 and then rapidly decreased (see Table 2). In contrast, the total body burden within the pups continued to rise until the pups were weaned. Between PND15 to PND25 the total body burden showed only a slight increase and the rapid rise in body weight apparently resulted in the substantial decrease in the tissue concentration.

Partial results of the disposition of TCDD within individual tissues are presented in Table 3. Examination of individual tissues apparently demonstrates that lactational transfer, as measured by pup stomach contents, was highest on postnatal day 1 and decreased thereafter; it should be noted that the volume of milk production was not determined and thus total transfer could not be calculated. Among other tissues, serum and reproductive tissues remained relatively constant until PND15, liver concentrations peaked at PND4 and adipose concentrations increased until PND15. Between PND15 and 25 all tissues showed rapid decreases between PNDs 15 and 25. For example, the concentration of TCDD within seminal vesicles decreased by approximately 80% between PNDs 15 and 25; this decrease was not a dilution of the tissue concentration as the total amount of TCDD within the seminal vesicles dropped from 1.5 to 0.6 pg.

These data define the tissue concentration of TCDD within the dam and offspring during a critical period of development and help to further establish TCDD tissue concentration associated with developmental alterations.

Table 1. Disposition in Maternal Tissues

PND	Blood	Liver	Skin	Adipose	Liver/Fat
1	.010±.0010	3.378±.425	.211±.017	1.513±.094	2.232
4	.008±4.0x10^{-4}	2.221±.375	.204±.017	1.703±.099	1.304
7	.004±4.0x10^{-4}	.892±.122	.143±.010	1.295±.101	.689
15	.005±.001	.351±.123	.094±.010	1.250±.143	.281
25	.002±3.0x10^{-4}	.157±.052	.058±.008	.550±.065	.284

Table 2. Disposition in Whole Male Pups

PND	%dose/g	ng TCDD/g	%dose/pup	ng TCDD/pup	Body Weight
1	.0400±8.99x10^{-4}	.108±3.41x10^{-3}	.224±3.45x10^{-3}	.605±.024	5.6±0.1
4	.113±.012	.303±.031	.975±.172	2.61±.452	8.4±0.9
7	.111±.013	.292±.034	2.01±.204	5.29±.523	18.3±0.6
15	.106±8.83x10^{-3}	.280±.024	3.40±.254	8.96±.607	32.6±2.9
25	.0501±3.45x10^{-3}	.133±.011	3.61±.17	9.53±.562	72.5±3.3

Table 3. Distribution of TCDD in Tissues of Male Offspring (ng/g)

PND	1	4	7	15	25
Serum	.012±.001	.017±.003	.017±.002	.021±.003	.004±.0003
Liver	.484±.090	2.573±.339	2.319±.335	1.842±.394	.805±.073
Stomach	.797±.083	.541±.062	.429±.036	.154±.019	.002±.001
Adipose	ND	.095±.008	.138±.010	.308±.082	.073±.009
Testis	.058±.010	.044±.003	.032±.004	.030±.004	.010±.001
Epididymis	.107±.025	.075±.005	.075±.017	.089±.011	.037±.004
Seminal Vesicle	ND	.158±.036	.155±.041	.166±.029	.030±.011
Prostate	ND	ND	.106±.015	.106±.012	.025±.005

ND= not determined

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