Deposition of PCDD/Fs and Non-ortho PCBs in Long Evans Rats

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Introduction

Developmental studies have shown that rat pups have delayed puberty and decreased sperm production after prenatal exposure to low levels of 2,3,7,8-TCDD which do not cause overt toxicity in adult rats.[1,2] Malformation of the external genitals in female pups was also observed.[3] Hurst et al.[4,5] administered pregnant Long Evans (LE) rats with a single dose of 0.05–1.15 ug [^{3}H]-TCDD/kg on Gestation Day (GD) 8 or GD 15 and measured maternal and fetus tissue concentrations on GD 9, GD 16, and GD 21. These results suggested how maternal and fetal tissue concentrations of TCDD relate to a biological response. However, the deposition of other dioxin-like compounds is still unclear. The purposes of our studies are (1) to determine the distribution of nine dioxin-like compounds—2,3,7,8-TCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDD, 1,2,3,7,8-PeCDF (1-PeCDF), 2,3,4,7,8-PeCDF (4-PeCDF), OCDF, 3,3',4,4'-TCB (PCB #77), 3,3',4,4',5-PeCB (PCB #126), and 3,3',4,4',5,5'-HxCB (PCB #169), in maternal rats and the offspring, (2) to relate the measured concentrations to the developmental and reproductive effects, and (3) to compare the adverse effects caused by the nine compounds to those caused by TCDD at the same TEQ levels.

Materials and Methods

Pregnant LE rats received a single dose (containing the nine previously mentioned dioxin-like compounds) of 0.0 (control), 0.05, 0.2, 0.8, or 1.0 ug TEQ/kg body weight in 5.0 mL corn oil/kg by oral gavage on GD 15. The doses of the nine compounds (ng/kg rat body) were as follows:

Dosing level ug TEQ/kg	TCDD ng/kg	TCDF ng/kg	PeCDD ng/kg	1-PeCDF ng/kg	4-PeCDF ng/kg	OCDF ng/kg	PCB 77 ng/kg	PCB 126 ng/kg	PCB 169 ng/kg
0.05	7.36	6.10	5.78	1.93	7.16	27.1	672	244	128
0.2	29.8	23.8	21.4	7.61	27.8	106	2750	976	516
0.8	118	103	89.4	31.7	116	442	11400	4110	2180
1.0	145	128	115	39.2	146	575	13300	4950	2710

4 females per dosage were sacrificed on GD 16, GD 21 and postnatal day 4 (PND 4) and five rat tissues were collected for chemical analysis—maternal serum, liver, and adipose tissue, placentas, and whole fetuses (pups). The details of the analytical method will be reported elsewhere. In short, serum was mixed with formic acid and was extracted with *n*-heptane. The other four tissues were ground with anhydrous sodium sulfate, sonicated with acetonitrile, and followed by solid-phase extraction (SPE) with C18 cartridges. The extracts of both serum and the

solid tissues were then cleaned up with 40% H_2SO_4-coated silicic acid and activated acidic alumina. Collected eluents were analyzed by GC-HRMS.

In addition to the chemical analysis, several biological end-points, such as induction of 7-ethoxyresorufin *o*-deethylase (EROD), sperm counts of male pups, and offspring body weight, were also surveyed (the details will be presented elsewhere).

Results and Discussion

We have obtained the concentrations of dioxin-like compounds in GD 16 serum and GD 16 fetus when we submitted this abstract. Table 1 lists the recoveries (mean ± SD, %) of ^{13}C-labeled internal standards. Table 2 and Table 3 shows the tissue concentrations (mean ± SD, pg/g) of the nine dioxin-like compounds. Table 4 and Table 5 reports the ratios (× 100) of the measured concentrations in serum and fetus to the dosing concentrations that maternal rats received. Five data points, the concentrations of TCDD/F, PeCDD, PeCB, and HxCB of one GD 16 serum sample at the 0.8 ug TEQ/kg dosage, were dropped. They were removed based on four criteria: (1) they seemed to be outliers on the scatter diagrams of dose–serum concentration (not enclosed in this abstract); (2) they lay as far as 2.5 or more standard deviations from mean values; (3) their jackknife residuals were larger than the critical value at α=0.05; (4) their Cook's distances were smaller than 1.0 so that the regression models of dose–tissue concentration would not change dramatically after deleting these points. Before these points were dropped, the raw data of this sample were scrutinized again to ensure that the outliers did not result from wrong calculations or improper experimental procedures. Similarly, one point of TCDF at the 0.8 ug TEQ/kg dosing level and one point of OCDF at the 1.0 ug TEQ/kg dosage were dropped from the GD 16 fetus data. Totally there were 5 of 141 serum data points and 2 of 129 fetus data points were not used.

Sub-ppt levels of 3,3',4,4'-TCB were detected in serum as well as in fetuses of the control group. It was not surprising to find dioxin-like compounds in control rat tissues. Vanden Heuvel et al.[6] also reported that PCDD/Fs were found in standard laboratory feed and in the liver of untreated rats.

When a straight-line model was applied to the data of GD 16 serum at 0.05–0.8 ug TEQ/kg, the squares of the correlation coefficients (r^2) between doses and serum concentrations were 0.71–0.98 for the nine compounds. Consequently, the relationship between doses and serum concentrations is linear from 0.05 to 0.8 ug TEQ/kg. The linearity was also observed on the scatter diagrams of dose–serum concentration. However, the slope of serum concentrations versus doses of every compound turned negative from 0.8 to 1.0 ug TEQ/kg dosage. The mean concentration of each compound in serum at the dose of 1.0 ug TEQ/kg was lower than that at 0.8 ug TEQ/kg dosing level, although there were no statistical differences (p-values are 0.23–0.96) between these two dosages. The r^2 declined to 0.62–0.91 when the 1.0 ug TEQ/kg data were included in the models. Diliberto et al.[7] described that there was increased hepatic sequestration of TCDD at higher doses because of induction of the binding protein CYP1A2 in liver. This may explain why the serum concentrations were lower at the 1.0 ug TEQ/kg than those at 0.8 ug TEQ/kg. Likewise, the relationship between doses and fetal concentrations was linear from 0.05 to 0.8 ug TEQ/kg. Between 0.8 to 1.0 ug TEQ/kg, the fetal concentrations of TCDD and the three PCBs increased in proportion to dosages. However, other five compounds increased much more than would be expected. The binding sites of the dioxin-like compounds in maternal rats may be saturated at 1.0 ug TEQ/kg dosage and a larger portion of these compounds entered fetuses. This could be another reason why the maternal serum concentrations of the compounds to be lower at 1.0 ug TEQ/kg than at 0.8 ug TEQ/kg.

The doses of TCDD in our study were 7.4–145 ng/kg body weight. The ratios of TCDD concentrations in GD 16 serum to the dosing concentrations were 0.020–0.091 and those in GD 16 fetus were 0.046–0.064. These findings were comparable to the results of Hurst et al.[5], which were 0.033–0.051 in GD 16 maternal blood and 0.049–0.11 in GD 16 whole fetuses, although their dosing concentrations were much higher (0.050–1.0 ug TCDD/kg body). Because we administered the rats with a mixture of the nine compounds (not only TCDD) at 0.050–1.0 ug TEQ/kg body, the effect of hepatic sequestration may be similar to that of TCDD only. This may clarify why the results of the two studies are alike.

We will obtain the concentrations of nine dioxin-like compounds in five different tissues of rats, at three different time-points: (1) GD 16, 24 hours after dosing; (2) GD 21, the last day of pregnancy; (3) PND 4, four days after the birth of pups. These results will provide a better understanding of toxicokinetics of the dioxin-like compounds. The relationship between the compound concentrations in the tissues and the adverse effects on the offspring will help to access the potential risks of human exposure to the dioxin-like compounds.

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Table 1. The recoveries (mean ± SD, %) of ^{13}C internal standards of GD 16 serum and GD 16 fetus samples (n=21)

GD 16 tissues	TCDD	TCDF	PeCDD	1-PeCDF	4-PeCDF	OCDF	PCB 77	PCB 126	PCB 169
Serum	98 ± 17%	115 ± 24%	96 ± 15%	104 ± 16%	99 ± 14%	88 ± 22%	104 ± 20%	96 ± 17%	91 ± 13%
Fetus	68 ± 18%	71 ± 18%	68 ± 15%	71 ± 15%	70 ± 14%	62 ± 11%	63 ± 17%	71 ± 18%	68 ± 15%

Table 2. The concentrations (mean ± SD, pg/g) of GD 16 serum samples (n=4; * n=3).
ND = not detectable

Dosing level ug TEQ/kg	TCDD pg/g	TCDF pg/g	PeCDD pg/g	1-PeCDF pg/g	4-PeCDF pg/g	OCDF pg/g	PCB 77 pg/g	PCB 126 pg/g	PCB 169 pg/g
0.0 (control)	ND	ND	ND	ND	ND	ND	0.033 ± 0.042	ND	ND
0.05	0.670 ± 0.081	0.111 ± 0.031	0.505 ± 0.076	ND	0.049 ± 0.027	0.275 ± 0.146	5.58 ± 3.57	18.9 ± 5.3	15.0 ± 4.5
0.2	1.36 ± 0.20	0.253 ± 0.076	0.690 ± 0.070	0.030 ± 0.014	0.072 ± 0.027	0.472 ± 0.151	7.62 ± 1.97	49.5 ± 12.3	37.6 ± 10.5
0.8	3.37 ± 0.31*	1.28 ± 0.09*	1.42 ± 0.11*	0.097 ± 0.034	0.308 ± 0.068	1.53 ± 0.68	20.2 ± 3.9	127 ± 15*	97.6 ± 11.8*
1.0	2.91 ± 0.83	1.06 ± 0.26	1.18 ± 0.30	0.076 ± 0.032	0.244 ± 0.088	1.38 ± 0.54	22.0 ± 6.5	126 ± 30	101 ± 17

Table 3. The concentrations (mean ± SD, pg/g) of GD 16 fetus samples (n=4; * n=3).
ND = not detectable

Dosing level ug TEQ/kg	TCDD pg/g	TCDF pg/g	PeCDD pg/g	1-PeCDF pg/g	4-PeCDF pg/g	OCDF pg/g	PCB 77 pg/g	PCB 126 pg/g	PCB 169 pg/g
0.0 (control)	ND	ND	ND	ND	ND	ND	0.116 ± 0.101	ND	ND
0.05	0.472 ± 0.033	0.031 ± 0.010	0.032 ± 0.003	ND	0.018 ± 0.002	ND	2.45 ± 1.82	11.3 ± 0.84	5.61 ± 0.47
0.2	1.76 ± 0.49	0.117 ± 0.036	0.138 ± 0.029	0.012 ± 0.002	0.060 ± 0.022	ND	3.70 ± 1.40	27.7 ± 5.7	17.0 ± 3.3
0.8	5.45 ± 1.06	0.616 ± 0.021*	0.691 ± 0.097	0.025 ± 0.011	0.216 ± 0.093	0.069 ± 0.011	8.02 ± 1.31	72.0 ± 4.8	49.9 ± 3.9
1.0	8.11 ± 0.84	1.08 ± 0.11	1.88 ± 0.34	0.254 ± 0.092	1.15 ± 0.17	0.344 ± 0.048*	12.4 ± 3.1	83.8 ± 8.2	57.9 ± 6.5

Table 4. The ratio (× 100) of concentrations in GD 16 serum samples to the dosing concentrations (n=4; * n=3). NA = not available

Dosing level ug TEQ/kg	TCDD	TCDF	PeCDD	1-PeCDF	4-PeCDF	OCDF	PCB 77	PCB 126	PCB 169
0.05	9.1 ± 1.1	1.8 ± 0.5	8.7 ± 1.3	NA	0.69 ± 0.38	1.0 ± 0.5	0.83 ± 0.53	7.8 ± 2.2	11.7 ± 3.5
0.2	4.6 ± 0.7	1.1 ± 0.3	3.2 ± 0.3	0.39 ± 0.19	0.26 ± 0.10	0.45 ± 0.14	0.28 ± 0.07	5.1 ± 1.3	7.3 ± 2.0
0.8	2.8 ± 0.3*	1.2 ± 0.1*	1.6 ± 0.1*	0.31 ± 0.11	0.27 ± 0.06	0.35 ± 0.15	0.18 ± 0.03	3.1 ± 0.4*	4.5 ± 0.5*
1.0	2.0 ± 0.6	0.83 ± 0.21	1.0 ± 0.3	0.20 ± 0.08	0.17 ± 0.06	0.24 ± 0.09	0.17 ± 0.05	2.5 ± 0.6	3.7 ± 0.6

Table 5. The ratio (× 100) of concentrations in GD 16 fetus samples to the dosing concentrations (n=4; * n=3). NA = not available

Dosing level ug TEQ/kg	TCDD	TCDF	PeCDD	1-PeCDF	4-PeCDF	OCDF	PCB 77	PCB 126	PCB 169
0.05	6.4 ± 0.4	0.51 ± 0.16	0.55 ± 0.05	NA	0.26 ± 0.03	NA	0.36 ± 0.27	4.6 ± 0.3	4.4 ± 0.4
0.2	5.9 ± 1.6	0.49 ± 0.15	0.64 ± 0.14	0.16 ± 0.03	0.22 ± 0.08	NA	0.13 ± 0.05	2.8 ± 0.6	3.3 ± 0.6
0.8	4.6 ± 0.9	0.60 ± 0.02*	0.77 ± 0.11	0.08 ± 0.04	0.19 ± 0.08	0.016 ± 0.004	0.071 ± 0.011	1.8 ± 0.1	2.3± 0.2
1.0	5.6 ± 0.6	0.84 ± 0.09	1.6 ± 0.3	0.65 ± 0.24	0.79 ± 0.12	0.060 ± 0.008*	0.093 ± 0.023	1.7 ± 0.2	2.1 ± 0.2