TIME-COURSE TRANSFER OF PCDD/FS AND NON-ORTHO PCBS TO FETAL AND NEONATAL LONG EVANS RATS

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Introduction

Limited pharmacokinetic information on the transfer of dioxins, furans, and PCBs to the fetus, placenta, and pup exists for congeners other than TCDD. In addition, pharmacokinetic properties among dioxin-like compounds may be significantly different. For example, the half-lives of 2,3,7,8-TCDF and 1,2,3,7,8-PeCDF (1-PeCDF) in rats are much shorter than that of 2,3,7,8-TCDD and 2,3,4,7,8-PeCDF (4-PeCDF)[1]. DeVito and Birnbaum demonstrated that pharmacokinetics can play an important role in relative potency of toxicants[2]. Furthermore, TCDD and other dioxin-like compounds normally exist in the environment as complex mixtures. Our objectives were to investigate the transfer of nine dioxin-like compounds to fetus, pup, and placenta from pregnant Long Evans (LE) rats, using a dosing solution that simulated the relative abundance of these compounds in food[3].

Methods and Materials

Chemicals. 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), and OCDF were purchased from Ultra Scientific (North Kingstown, RI, USA; purity > 98%). 3,3',4,4'-TCB (PCB77), 3,3',4,4',5-PeCB (PCB126), and 3,3',4,4',5,5'-HxCB (PCB169) were purchased from Accustandard (New Haven, CT, USA; purity > 99%). $^{13}C_{12}$-labeled PCDD/Fs and PCBs were from Cambridge Isotope Laboratories (Andover, MA, USA; purity 99%).

Animals and treatment. Pregnant Long Evans (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 Gestation Day (GD) 15. The doses of the nine compounds (ng/kg rat body) were as follows and the TEQ of the dosage was calculated according to the 1998 WHO TEFs[4]:

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

Four females per dosage were sacrificed on GD 16, GD 21 (the last day of pregnancy) and fetuses and placentas were collected for chemical analysis using high-resolution gas chromatography (HRGC)/high-resolution mass spectrometry (HRMS) and an isotope-dilution technique[5]. On postnatal day 4 (PND 4), four pups (from four different dams) per dosage were also analyzed.

Statistical Analysis. Data among dosing groups were compared using SAS 6.12 for windows with Tukey's multiple comparison at $\alpha = 0.05$. When the cell-specific sample sizes were not equal, Scheffe's method was used.

Results and Discussion

Significant dose-dependent decrease in %dose/g was seen with placenta (Table 1 and 2), despite the high correlation between the placenta concentration (pg/g) and the dose ($r = 0.82–0.97$, Table 4). There was also a dose-dependent decrease of %dose in GD 21 fetus and PND 4 pup (Table 2 and 3) which may be due to the dose-dependent increase in liver sequestration due to the induction of CYP1A2[6].

On GD 16, the %dose/g fetal tissue of dioxins and furans were similar among 0.05, 0.2, and 0.8 ug TEQ/kg levels. However, the concentrations increased abruptly at 1.0 ug TEQ/kg except for TCDD (Table 1). This phenomenon was not seen with the three co-planar PCBs, whose dosages were much higher than those of PCDD/Fs in our study.

Transfer of dioxin-like compounds through milk is much higher than via placenta[7,8]. After suckling milk for four days, PND 4 pups had five to 25 times greater concentrations than that of GD 21 fetus for most compounds (Table 2 and 3). The %dose/g pup decreased as the dosage increased, especially for TCDF, 1-PeCDF, and PCB 77(Table 3). The correlation coefficients between dose and pup concentration (pg/g tissue) of TCDF and PCB 77 were negative (Table 4). This may be due to autoinduction of metabolism, which is only obvious at high doses[9]. Because TCDF, 1-PeCDF, and PCB77 are metabolized and eliminated more quickly than other 2,3,7,8-substituted congeners[1,11,12,13], the autoinduction effect would be more apparent with them. The low %dose of the three compounds in GD 21 fetus and placenta (Table 2) also implied their rapid excretion by the dams.

DeVito et al. showed that 4-PeCDF has higher hepatic affinity than PeCDD and TCDD[10] and this may explain why 4-PeCDF had the lowest %dose in fetus/pup and placenta among the three compounds. The low %dose of OCDF in tissues could be attributed to its poor GI absorption[14].

None of the dosed chemicals was detected in all batches of sample blanks (representing the level of reagent and laboratory contamination) except for PCB 77, which was from the heptane used in chemical analysis (about 4 fg/ml in the solvent). However, some analytes were found in the tissues of control rats. PCB 77 (ND–0.3 pg/g) was the only chemical detected in control GD 16 fetuses. PCB 77, 126, and 169 were observable in control GD 21 fetuses (ND–0.9 pg/g) and PND 4 pups (ND–2.9 pg/g). In the control GD 16 placenta, dioxins, furans and PCB 77 ranged

from ND to 1 pg/g whereas PCB 126 and PCB 169 ranged from ND to 17 pg/g. In contrast, only the three coplanar PCBs (ND–1.5 pg/g) were detected in control GD 21 placentas.

We also obtained the concentrations of the nine dioxin-like compounds in maternal liver, adipose tissue, and serum, at the three time-points, and data analysis is ongoing. These results will provide a better understanding of toxicokinetics of the dioxinlike compounds in pregnant animals.

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Table 1. The average concentration (%dose/g) of GD 16 fetus and placenta. The dosing levels were shown at the first column in ug TEQ/kg. ND = not detectable.

Fetus	TCDD	TCDF	PeCDD	1-PeCDF	4-PeCDF	OCDF	PCB 77	PCB 126	PCB 169
0.05	0.020	0.0016 [d]	0.0017 [d]	ND	0.00069 [d]	ND	0.00091	0.015 [b,c,d]	0.014 [b,c,d]
0.2	0.019	0.0015 [d]	0.0020 [d]	0.00051 [d]	0.00067 [d]	0.00015	0.00037	0.0089 [a,c,d]	0.010 [a,d]
0.8	0.015	0.0017 [d]	0.0025 [d]	0.00028 [d]	0.00064 [d]	0.000052	0.00021	0.0056 [a,b]	0.0074 [a]
1.0	0.018	0.0027 [a,b,c]	0.0051 [a,b,c]	0.0023 [b,c]	0.0025 [a,b,c]	0.00020	0.00029	0.0054 [a,b]	0.0068 [a,b]

Placenta	TCDD	TCDF	PeCDD	1-PeCDF	4-PeCDF	OCDF	PCB 77	PCB 126	PCB 169
0.05	0.060 [b,c,d]	0.022 [b,c,d]	0.024 [c,d]	0.034 [b,c,d]	0.010 [c,d]	0.0042	0.0032 [c,d]	0.023 [c,d]	0.032
0.2	0.050 [a,c,d]	0.015 [a,c,d]	0.025 [c,d]	0.017 [a,c,d]	0.0091 [c,d]	0.0043	0.0018	0.023 [c,d]	0.033 [d]
0.8	0.031 [a,b]	0.0060 [a,b]	0.015 [a,b]	0.0074 [a,b]	0.0049 [a,b]	0.0033	0.0010 [a]	0.014 [a,b]	0.022
1.0	0.029 [a,b]	0.0053 [a,b]	0.013 [a,b]	0.0074 [a,b]	0.0043 [a,b]	0.0033	0.00089 [a]	0.014 [a,b]	0.018 [b]

[a,b,c,d] Significantly different with 0.05, 0.2, 0.8, and 1.0 ug TEQ/kg dose groups, respectively.

Table 2. The average concentration (%dose/g) of GD 21 fetus and placenta. The dosing levels were shown at the first column in ug TEQ/kg. ND = not detectable.

Fetus	TCDD	TCDF	PeCDD	1-PeCDF	4-PeCDF	OCDF	PCB 77	PCB 126	PCB 169
0.05	0.053 [c,d]	ND	0.019 [c,d]	ND	0.0078	ND	0.00041	0.011 [c,d]	0.0094
0.2	0.043 [c,d]	0.0020	0.021 [c,d]	ND	0.0056 [c,d]	0.00090	0.00016	0.011 [c,d]	0.0092
0.8	0.028 [a,b]	ND	0.010 [a,b]	0.00076	0.0025 [b]	0.00017	0.000074	0.0069 [a,b]	0.0066
1.0	0.024 [a,b]	ND	0.010 [a,b]	ND	0.0026 [b]	0.00023	0.000059	0.0069 [a,b]	0.0065

Placenta	TCDD	TCDF	PeCDD	1-PeCDF	4-PeCDF	OCDF	PCB 77	PCB 126	PCB 169
0.05	0.043 [c,d]	ND	0.026 [c,d]	ND	0.0098 [c,d]	0.0052 [c,d]	0.00078	0.013	0.016
0.2	0.036 [c]	ND	0.025 [c,d]	0.014 [c,d]	0.0068 [a,b]	0.0032	0.00056	0.020 [c,d]	0.023 [c,d]
0.8	0.022 [a,b]	ND	0.014 [a,b]	0.0025 [b]	0.0024 [a,b]	0.0019 [a]	0.00027	0.011 [b]	0.015 [b]
1.0	0.024 [a]	ND	0.014 [a,b]	0.0045 [b]	0.0025 [a,b]	0.0019 [a]	0.00024	0.011 [b]	0.013 [b]

[a,b,c,d] Significantly different with 0.05, 0.2, 0.8, and 1.0 ug TEQ/kg dose groups, respectively.

Table 3. The average concentration (%dose/g) of PND 4 pups. The dosing levels were shown at the first column in ug TEQ/kg.

Pup	TCDD	TCDF	PeCDD	1-PeCDF	4-PeCDF	OCDF	PCB 77	PCB 126	PCB 169
0.05	0.32 [b,c,d]	0.053 [b,c,d]	0.25 [b,c,d]	0.060 [b,c,d]	0.092 [b,c,d]	0.0041	0.0053	0.28 [b,c,d]	0.24 [c,d]
0.2	0.20 [a]	0.0030 [a]	0.16 [a,d]	0.0083 [a]	0.037 [a,d]	0.0028	0.00022 [a]	0.18 [a,c,d]	0.19
0.8	0.12 [a]	0.00051 [a]	0.097 [a]	0.0042 [a]	0.021 [a]	0.0027	0.000045 [a]	0.10 [a,b]	0.12 [a]
1.0	0.12 [a]	0.00042 [a]	0.078 [a,b]	0.0033 [a]	0.016 [a,b]	0.0022	0.000047 [a]	0.088 [a,b]	0.10 [a]

[a,b,c,d] Significantly different with 0.05, 0.2, 0.8, and 1.0 ug TEQ/kg dose groups, respectively.

Table 4. Correlation coefficient of dose (ng) vs concentration (pg/g tissue) of fetus/pup and placenta. NA = not available.

	TCDD	TCDF	PeCDD	1-PeCDF	4-PeCDF	OCDF	PCB 77	PCB 126	PCB 169
GD 16 fetus	0.966	0.935	0.911	0.683	0.810	0.714	0.857	0.977	0.977
GD 16 placenta	0.964	0.894	0.933	0.862	0.933	0.862	0.875	0.948	0.952
GD 21 fetus	0.942	NA	0.899	NA	0.856	0.427	0.685	0.924	0.924
GD 21 placenta	0.954	NA	0.968	0.404	0.881	0.972	0.819	0.946	0.947
PND4 pup	0.896	-0.682	0.860	0.461	0.850	0.773	-0.498	0.884	0.879