A MIXTURE OF POLYCHLORINATED DIBENZO-*P*-DIOXINS (PCDDS), DIBENZOFURANS (PCDFS), AND NON-*ORTHO* POLYCHLORINATED BIPHENYLS (PCBS) CHANGED THE LIPID CONTENT OF PREGNANT LONG EVANS RATS

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Introduction

2,3,7,8-TCDD increases serum cholesterol and triglyceride concentrations in both dioxin-exposed workers and experimental animals[1,2]. 2,3,4,7,8-PeCDF and 3,3',4,4',5-PeCB (PCB 126) elevate serum triglycerides in rhesus monkeys and guinea pigs, but decrease plasma cholesterol in rhesus monkeys and Wistar rats[3,4].

TCDD and 1,2,3,7,8-PeCDD cause liver hypertrophy and raise liver triglycerides and total lipid[2,5,6,7]. PCB 126 and 3,3',4,4',5,5'-HxCB (PCB 169) have the same effects on liver as TCDD but at a higher dosage[4,8].

Although numerous studies have reported TCDD effects on the lipid content of liver and serum, limited information about the lipid content of fetus, pup and placenta is available. In addition, most studies have used extremely high doses of TCDD compared with the environmental level. Dioxin-like compounds also normally exist in the environment as complex mixtures. Consequently, our objectives were to investigate the effect of a low-dose exposure to a mixture of dioxins, furans, and PCBs, which simulated the relative abundance of these compounds in food[9], on the lipid content of pregnant Long Evans (LE) rat tissues, especially on fetus, pup, and placenta. The information is desirable for future studies because lipid content is used to compare and normalize TCDD/TEQ values.

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'-tetrachlorobiphenyl (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%). The TEQ of the dosing solution was calculated according to the 1998 WHO TEFs[10].

Animals and treatment. Time-pregnant Long Evans (LE) rats (gestational day 9/ day after mating = GD 0) were obtained from Charles River Breeding Laboratories (Raleigh, NC, USA). Females were housed in plastic cages containing heat-treated pine shavings (Beta Chips, North Eastern Products Inc., Warrensburg, NY, USA) and given food (Purina 5001 Rodent Chow, Ralston Purina Co., St. Louis, MO, USA) and water *ad libitum.* On GD 15, rats received a single dose (Table 1) of 1.0 ug TEQ/kg body weight in 5.0 mL corn oil/kg by oral gavage and the control group received vehicle alone (rats weighted 316.6 ± 23.0 g on GD 15). GD 16 fetus and placenta, GD 21 (the last day of pregnancy) fetus and placenta, postnatal day 4 (PND 4) pup, and PND 4 maternal liver/adipose/serum were collected for determination of lipid content.

Determination of lipid content. A modified method of Folch *et al.* was utilized[11]. Briefly, tissues were blended in 2:1 chloroform–methanol mixture (v/v) twice. The extracts were filtered through glass fiber and combined. The filtrate was mixed with water, which resulted in a mixture of chloroform/methanol/water (8:4:3, v/v/v). The lower phase of the mixture was collected, evaporated to dryness, and the lipid content was determined gravimetrically.

Statistical analysis. Data were analyzed with two-tailed *t*-test ($p < 0.05$).

Results and Discussion

The lipid content of the treated fetuses was identical to that of the control group on both GD 16 and GD 21 (Table 2). In contrast, just four days after birth, the treated PND 4 pups had 1.3-fold greater lipid content than that of the controls (Table 2). Studies have shown that transfer of dioxin-like compounds through milk is much higher than via placenta[12,13]. The transferred compounds may reduce lipid clearance of pups by altering the apoprotein composition of mesenteric lymph chylomicrons and very low density lipoprotein (VLDL), which are needed for moving absorbed lipid from the enterocytes into the lymph and for transporting lipid out of cells respectively[14]. The compounds may also diminish lipid catabolism by decreasing β-oxidation of fatty acids[15].

The lipid content of the treated GD 16 and GD 21 placenta dropped 50% and 44% respectively, as compared with the control group (Table 2). Nevertheless, there was no significant difference in the individual placenta weight between the two groups [GD 16: 0.248 ± 0.039 g (control) vs 0.254 ± 0.008 g (treated); GD 21: 0.426 ± 0.048 g (control) vs 0.562 ± 0.133 g (treated)]. The lipid content of the liver and serum from treated dams was 76% and 62% of the control group (Table 2) respectively, but the liver weight of the treated dams was not significantly different from the controls (treated: 16.67 ± 2.16; control: 16.04 ± 1.71). The lipid content of the adipose tissue from treated maternal rats was not significantly different from controls (Table 2).

Lakshman *et al.* reported that TCDD-treated rats had lower acetyl-coenzyme A (CoA) carboxylase, fatty acid synthase, and 3-hydroxy-3-methylglutaryl-CoA reductase activities so that the synthesis rates of fatty acid and cholesterol in liver and adipose tissue were decreased[16]. This alteration in synthesis may explain why the treated group had lower lipid content in liver, serum, and placenta. Ishimura *et al.* demonstrated that TCDD altered placental growth and function[17]. Decreased growth would also contribute to the lower placental lipid content in the treated group.

Our study demonstrated that a low-dose mixture of dioxin-like compounds changes lipid content in the maternal liver, serum, and in the placenta. In contrast, the offspring were not affected until they got high exposure via lactation.

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TABLE 1. The dosage of compounds (ng/kg rat body), which composed about 1.0 ug TEQ/kg Body Weight. [a,b]

TCDD	TCDF	PeCDD	1-PeCDF	4-PeCDF	OCDF	PCB 77	PCB 126	PCB 169
145	128	115	39.2	146	575	13300	4950	2710

[a] The Relative Concentrations Approximated the Relative Abundance of These Compounds in a Food Mixture (Birnbaum & DeVito, 1995).
[b] The TEQ was calculated according to the 1998 WHO TEFs (Van den Berg *et al.*, 1998).

TABLE 2. Lipid Content (%) of Long Evans Rat Tissues (Mean ± Standard Deviation, n = 4)

	GD 16 Fetus	GD 16 Placenta [a]	GD 21 Fetus	GD 21 Placenta [a]	PND 4 Pup [a]	PND 4 Maternal Liver [a]	PND 4 Maternal Adipose	PND 4 Maternal Serum [a]
Control	1.14 ± 0.01	4.02 [b] ± 0.17	1.37 ± 0.05	4.02 [b] ± 0.15	5.68 ± 0.69	6.51 ± 0.22	86.1 ± 4.6	0.71 ± 0.15
Treated	1.16 ± 0.07	2.01 ± 0.10	1.37 ± 0.23	2.26 ± 0.04	7.51 ± 1.30	4.92 ± 0.69	82.2 ± 2.9	0.44 ± 0.09

[a] Significantly different between control and treated groups ($p < 0.05$) by two-tailed *t*-test.
[b] n = 3.