The effects of mixtures of PCDDs, PCDFs, and PCBs on hepatic retinyl palmitate concentrations in mice.

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Introduction

Human exposure to polyhalogenated aromatic compounds occurs as complex mixtures of PCDDs, PCDFs and PCBs[1-3]. In experimental animals exposure to individual PCDDs, PCDFs, and PCBs elicits a number of toxic responses depending upon the chemical[1-3]. A number of the PHAHs induce toxic responses through binding to the Ah receptor[1-3]. Estimates of potential health risks due to exposure to mixtures of PHAHs have employed the TEF methodology[1-3]. The use of the TEF methodology assumes that the relative potency of a chemical is consistent across endpoints and that the response elicited by a complex mixture is dose additive. This methodology only examines the risk of a select group of the PHAHs which bind to the Ah receptor.

Exposure of rats to TCDD and related compounds has been associated with changes in levels of retinol (Vitamin A) and its ester derivatives in tissues[4-6]. In the present study, the effect of TCDD and a mixture of PCDDs, PCDFs, and PCBs on hepatic retinol and retinyl palmitate, the main storage form of Vitamin A in the livers of mice, were examined. In addition to dioxinlike PCBs, the present study examines the interactions with a non-dioxinlike PCB, 2,2',4,4',5,5'-hexachlorobiphenyl. These studies indicate that TCDD decreases retinyl palmitate in hepatic tissue of mice and that the TEF methodology adequately estimates the relative potency of a complex mixture of dioxinlike PHAHs. In contrast, PCB 153 increases the concentration of retinyl palmitate in hepatic tissue and when administered with either TCDD or a mixture of PCDDs, PCDFs, and PCBs, it attenuates the dioxinlike effects on retinyl palmitate.

Material and Methods

Chemicals: TCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PCDD), 2,3,7,8-

tetrachlorodibenzofuran (TCDF), 1,2,3,7,8-pentachlorodibenzofuran (1PCDF), 2,3,4,7,8-pentachlorodibenzofuran (4PCDF), and octachlorodibenzofuran (OCDF) were purchased from Ultra Scientific (purity >98%). 3,3',4,4'-Tetrachlorobiphenyl (PCB 77), 3,3',4,4',5-pentachlorobiphenyl (PCB 126), 3,3',4,4',5,5'-hexachlorobiphenyl (PCB 169), 2,3,3',4,4'-pentachlorobiphenyl (PCB 105), 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 2,2',4,4',5,5'-hexachlorobiphenyl (153) and 2,3,3',4,4',5-hexaclorobiphenyl (PCB 156) were purchased from Accu Standard, New Haven, CT (purity > 98%). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Animals and treatment: Female B6C3F1 mice (60 days old) were obtained from Charles River Breeding Laboratories, Raleigh, NC. Water and food were given *ad libitum*. The animals were held under controlled conditions of temperature (22°C ± 1) and lighting (12/12 light/dark cycle). Mice were randomly assigned to treatment groups (7-9 per group) and group housed. Animals were dosed by gavage with corn oil solutions of the test chemicals 5 days a week for 13 weeks. The animals were exposed to A: TCDD alone (0.45, 1.5, 15, and 150 ng TCDD/kg/day); B: PCDDs, PCDFs, and PCBs (see table 1) (0.45, 1.5, 15, and 150 ng TEQ/kg/day); C: PCB 153 (7, 23, 232, 2323 ug 153/kg/d); D TCDD (0.45, 1.5, 15, 150 ng TCDD/kg/d) and PCB 153 (7.0, 23, 232, and 2323 ug 153/kg/d); E:The same mixture as in B plus PCB 153 at the same dose combinations in D. The composition of the mixture is shown in Table 1. Three days after the last dose, animals were killed. Livers were removed and S-9 fractions were prepared as described[7]. Total liver whole homogenate fractions were taken and frozen at -70°C until analysis.

Vitamin A analysis: Liver retinoids were extracted from 100 µl aliquots of liver homogenates with 250 µl of ethyl acetate containing 0.1% BHT as an antioxidant. The samples were then centrifuged at 15,000 rpm for 1-2 minutes. Two hundred microliters of the ethyl acetate layer was removed and evaporated using the speedvac at full vacuum, medium chamber heat (43°C) for 20 minutes. Each sample was resuspended with 250 µl of mobile phase (95% methanol/ 5% ethyl acetate) and a 200 µl aliquot was removed for analysis. Extraction efficiencies were routinely above 80%.

Twenty-five-microliters of the 200 µl sample were analyzed with HPLC using a C-18 reverse-phase analytical column (Rainin Microsorb ODS, 4.6 mm x 25 cm, 5 µm particle size) with a guard column (Ultrasphere ODS, 4.6 mm x 4.5 cm, 5 µm particle size). A Beckman HPLC System Gold. A wavelength of 326 nm with a 4 nm bandwidth was used for the detection of retinoids. The ambient gradient elution begins with 90:10 Methanol:Ethyl Acetate with a flow rate of 1 mL/min. The solvent gradient (90% to 70% Methanol) begins at 4 minutes and takes a period of 2 minutes. At 12 minutes, a 2 minute (70% to 90%) linear return begins reequilibrating at 90% methanol from 14 minutes to 20 minutes. A linear flow gradient (1 mL/min to 2 mL/min, and then back to 1 mL/min) occurs concurrently with the solvent gradient.

Statistical analysis: Retinol and retinyl palmitate levels were analyzed independently using a one-way analysis of variance (ANOVA) and the Fisher's PLSD ($p < 0.05$).

Results

The administration of any of the test chemicals or mixtures did not result in increased

mortality or morbidity, as measured by altered body weight gains, at any of the doses examined. In Table 2, the dose response relationship for alterations in retinyl palmitate for TCDD and the mixture is presented. TCDD decreased retinyl palmitate concentrations to 65% of control values at the highest dose examined. The administration of the TEQ mix also decreased retinyl palmitate concentrations in hepatic tissue, similar to that observed in the TCDD treated animals. For example, at 150 ng TCDD/kg/day, hepatic retinyl palmitate concentrations were decreased to 65 ± 10% of controls while 150 ng TEQ/kg/day decreased hepatic retinyl palmitate by to 59 ± 14% of controls (Table 2). PCB 153 significantly increased hepatic retinyl palmitate concentrations at the three lowest doses examined (Table 3). PCB 153 attenuated the decrease in retinyl palmitate at a combination of 15 ng TCDD/kg/d and 232 ug 153/kg/d but not at the highest dose combination examined (Table 2). Cotreatment with the TEQ mix (B) and PCB 153 did not alter the dose response for the TEQ mix (Table 2).

Discussion

Previous studies in female Sprague Dawley rats demonstrate that subchronic treatment with either TCDD or PCB 126 decreases hepatic retinol and retinyl palmitate concentrations[4,5]. The present study demonstrates that subchronic treatment with TCDD decreases hepatic retinyl palmitate concentrations in mice. Hepatic retinyl palmitate concentrations are also decreased in mice exposed to a complex mixture of PCDDs, PCDFs, and PCBs. The dose response relationship for the mixture was similar to that for TCDD indicating that the TEF methodology adequately predicted the relative potency of this mixture for decreases in retinyl palmitate. PCB 153 partially antagonized the effects of TCDD. However, this antagonism appears to be a physiological antagonism since PCB 153 produces the opposite effect on retinyl palmitate concentrations compared to TCDD. Physiological antagonism has been observed for the immunotoxic interactions of TCDD and PCB 153 in mice[10].

The relative potency of the complex mixture administered was estimated based on the relative enzyme (CYP1A1/1A2) induction potency of these congeners in female mice[8,9] The relative potency derived for enzyme induction accurately predicted the potency of this mixture to decrease retinyl palmitate concentrations. These results are consistent with earlier reports from this laboratory which demonstrate the ability of the relative potency estimates derived in mice in mice for enzyme induction to predict the relative potency of complex mixtures for enzyme induction and immunotoxicity[11]. These data demonstrate that the TEF methodology can be used to predict responses of complex mixtures and supports the use of TEFs in estimating human health risks

(This abstract does not necessarily represent USEPA policy.)

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Table 1
Chemical Composition and TEQs for 90-day PCDD, PCDF, and PCB mixture study

Chemical	Food Ratio	Relative potency values[a]		
		REPs[8,9]	TEQ	Total % TEQ
TCDD	1.0	1	1	42
PCDD	1.0	0.5	0.5	23
TCDF	1.5	0.01	0.02	<1
1PCDF	0.5	0.02	0.009	<1
4PCDF	2.0	0.09	0.2	8
OCDF	5.0	0.00006	0.0003	<1
77	150	0.0005	0.0008	<1
126	45	0.005	0.2	10
169	30	0.0004	0.01	<1
105	6000	0.0000003	0.002	<1
118	30000	0.00001	0.4	16
156	1000	0.00001	0.01	<1

[a] - Relative potency estimates are based on enzyme induction (CYP1A) in mice[8,9].

Table 2
Retinyl palmitate concentrations in liver of Female B6C3F1 mice after a 13-week Exposure to TCDD or after 13-week Exposure to Mixtures of PCDDs, PCDFs, and PCBs[a]

Dose (ng/kg/day)	TCDD	TCDD/153[c]	TEQ	TEQ/153[c]
0	2702 ± 230	2418 ± 217	2252 ± 154	3031 ± 213
0.45	2653 ± 351	2558 ± 196	2612 ± 220[b]	2765 ± 345[b]
1.5	2453 ± 327	2480 ± 109	2395 ± 295	2833 ± 327
15	2088 ± 127[b]	2245 ± 283	2106 ± 271	2881 ± 479
150	1763 ± 282[b]	1552 ± 118[b]	1335 ± 321[b]	1602 ± 239[b]

[a] data expressed as mean (ug/g tissue) ± standard deviation
[b] Significantly different form controls ($p < 0.05$)
[c] animals cotreated with either PCB 153 and either TCDD or the TEQ mixture.

Table 3
Retinyl palmitate concentrations in liver of Female B6C3F1 mice after a 13-week Exposure to PCB 153

Dose (ug/kg/day)	153
0	2966 ± 309
7	3568 ± 193[b]
23	3575 ± 673[b]
232	3782 ± 144[b]
2323	3357 ± 197

[a] data expressed as mean (ug/g tissue) ± standard deviation
[b] Significantly different form controls ($p < 0.05$)