Relative potency factors derived from CYP1A induction in mice are predictive for alterations in retinoid concentrations after subchronic exposure to mixtures of PCDDs, PCDFs, and PCBs in female Sprague Dawley rats

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Introduction

Polyhalogenated aromatic hydrocarbons (PHAHs) such as polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) are present in the environment as industrial compounds or industrial by-products. Often, these PHAHs are found as complex mixtures in the environment. Exposure to these compounds is known to cause a variety of toxic effects[1,2,3]. Toxic equivalency factors (TEFs) were developed to facilitate the calculation/estimate of the potency of complex mixtures[1]. Each individual compound is assigned a relative potency based on its potency compared to TCDD, the most potent of all dioxin-like compounds. In order to determine the toxic equivalence (TEQ) of each compound, the TEF for each individual compound is multiplied by the its concentration in the mixture. The summation of the TEQs provides the overall dioxin equivalent potency of the mixture.

The use of TEFs assumes that dose response curves are parallel for a given response between chemicals and that combined effects are additive. Previous studies have shown that TEFs adequately predict pulmonary and hepatic cytochrome P450 induction for mice and rats exposed to a mixture of PCDDs, PCDFs, and PCBs[4,5].

In this study, we tested the ability of TEFs to predict the effect on hepatic Vitamin A concentrations in female Sprague Dawley rats exposed to a mixture of PCDDs, PCDFs, and PCBs. The TEFs values used to calculate our TEQs were derived from 90 day studies in female B6C3F1 mice[6,7]. The concentrations in the mixture are representative of those found in food samples.

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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[8,9]. 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 liver, were examined.

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), 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 Sprague Dawley rats (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). Rats were randomly assigned to treatment groups (7 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, 4.5, 15, 45, 80, 150, and 450 ng/kg/day); or B: PCDDs, PCDFs, and PCBs (only planar and mono-ortho substituted PCBs) (0.45, 1.5, 15, and 150 ng TEQ/kg/day). 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[10]. 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 was used consisting of a 126 pump, 166 UV/Vis detector, and a 507 autosampler. 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 ($p < 0.05$).

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

Chemical	Food Ratio	Relative potency values		
		TEF[6,7]	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

Results

The administration of TCDD or the TEQ mix did not result in increased mortality or morbidity, as measured by altered body weight gains, at any of the doses examined. In Table 3, the dose response relationship for alterations in retinyl palmitate is presented. TCDD decreased retinyl palmitate concentrations to 26% 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 35 ± 8.4% of controls while 150 ng TEQ/kg/day decreased hepatic retinyl palmitate by to 40 ± 11% of controls. Hepatic retinol concentrations were not altered significantly by the administration of either TCDD or 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[11]. The present study confirms that subchronic treatment with TCDD decreases hepatic retinyl palmitate concentrations. Hepatic retinyl palmitate concentrations are also decreased in rats exposed to a complex mixture of PCDDs, PCDFs, and PCBs. In contrast, hepatic retinol concentrations were unaffected by administration of either TCDD or the TEQ mix. The reason for this discrepancy is uncertain. Van Birgelen and coworkers administered TCDD or PCB 126

in the diet[8] while the present study administered these compounds by corn oil gavage. It is possible that the means of administration effected the response to TCDD.

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[6,7]. The relative potency derived from mice accurately predicted the potency of this mixture in rats. Similar decreases in hepatic retinyl palmitate were observed in rats treated with either TCDD or the TEQ mix. These results are consistent with earlier reports from this laboratory which demonstrate the ability of the relative potency estimates derived in mice to predict enzyme induction in rats[5]. These data demonstrate that the TEF methodology can be used to predict responses of complex mixtures and supports the use of TEFs in extrapolation across species.

(This abstract does not necessarily represent USEPA policy.)

Literature

1) Birnbaum, L.S., and DeVito, M.J. (1995). Use of toxic equivalency factors for risk assessment for dioxins and related compounds. *Toxicology* **105**, 391-401.

2) Safe, S. (1990). Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *CRC, Crit. Rev. Toxicol.* **21**, 51-88.

3) Safe, S. (1994). Polychlorinated biphenyls (PCBs): Environmental impact, biochemical and toxic responses, and implications for risk assessment. *CRC, Crit. Rev. Toxicol.* **24**, 87-149.

4) Hurst, C., DeVito, M.J., Diliberto, J.J., and Birnbaum, L.S. (1995). Additive interactions of mixtures containing polychlorinated dibenzo-*p*-dioxins (PCDD, dibenzofurans (PCDF) and biphenyls (PCB). *The Toxicologist* **15**, 337.

5) Van Birgelen, A.P.J.M., DeVito, M.J., and Birnbaum, L.S. (1996). Toxic equivalency factors derived for cytochrome P450 induction in mice are predictive for cytochrome P450 induction after subchronic exposure to mixtures of PCDDs, PCDFs, and PCBs in female B6C3F1 mice and Sprague Dawley rats. *Organohalogen Compounds* **29**, 251-256.

6) Ross, D.G., DeVito, M.J., and Birnbaum, L.S. (1994). Relative induction potency of polychlorinated biphenyls (PCBs) and TCDD in mouse liver, lung, and skin. *The Toxicologist* **14**, 278.

7) DeVito, M.J., Menache, M.A., Ross, D.G., Diliberto, J.J., and Birnbaum, L.S. Relative potency of polyhalogenated dibenzo-*p*-doxins and dibenzofurans following subchronic treatment in mice: CYP1A1 and CYP1A2 enzyme activity in liver, lung, and skin. *Toxicol. Appl. Pharmacol* **(in press)**.

8) Van Birgelen, A.P.J.M., Van der Kolk, Fase, K.M., Bol, I., Poiger, H., Brouwer, A., Van den Berg, M. (1994). Toxic Potency of 3,3',4,4'5-Pentachlorobiphenyl Relative to and in Combination with 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in a Subchronic Feeding Study in the Rat. *Toxicol. Appl. Pharmacol.* **127**, 209-221.

9) DeVito, M.J., Jackson, J.A., Van Birgelen, A.P.J.M., and Birnbaum, L.S. (1997). Reduction in hepatic retinoid levels after subchronic exposure to dioxinlike compounds in female mice and rats. *The Toxicologist* **36**, 214.

10) DeVito, M.J., Maier, W.E., Diliberto, J.J., and Birnbaum, L.S. (1993). Comparative ability of various PCBs, PCDFs, and TCDD to induce cytochrome P450 1A1 and 1A2 activity following 4 weeks of treatment. *Fudam. Appl. Toxicol.* **20**, 125-130.

11) Van Birgelen, A.P.J.M., Van der Kolk, J., Fase, K.M., Bol, I., Poiger, H., Brouwer, A., and Van den Berg, M. (1995). Subchronic dose-response study of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in female Sprague Dawley rats. *Toxicol. Appl. Pharmacol.* **132,** 1-13.

Table 2
Retinol concentrations in liver of Female Sprague Dawley Rats after a 13-week Exposure to TCDD or after 13-week Exposure to Mixtures of PCDDs, PCDFs, and PCBs (mean ± SD)

Dose (ng/kg/day)	Retinol (TCDD) (ug per Gram Tissue)	Retinol (TEQ) (ug per Gram Tissue)
0	24.0 ± 5.0	32.0 ± 9.3
0.45	22.2 ± 4.7	41.5 ± 6.5
1.5	30.1 ± 7.5	48.3 ± 12.4[a]
4.5	25.0 ± 12.0	
15	21.0 ± 6.8	27.5 ± 6.8
45	30.8 ± 22.1	
80	22.5 ± 9.2	
150	20.7 ± 11.8	25.0 ± 9.2
450	17.7 ± 12.1	

[a] Significantly different form controls ($p < 0.05$)

Table 3
Retinyl Palmitate concentrations in liver of Female Sprague Dawley Rats after a 13-week Exposure to TCDD or after a 13-week Exposure to Mixtures of PCDDs, PCDFs, and PCBs (mean ± SD)

Dose (ng/kg/day)	Retinyl Palmitate (TCDD) (ug per Gram Tissue)	Retinyl Palmitate (TEQ) (ug per Gram Tissue)
0	1561.7 ± 232.8	1672.2 ± 235.5
0.45	1570.1 ± 93.6	1700.6 ± 117.8
1.5	1655.1 ± 164.4	1700.4 ± 185.1
4.5	1294.9 ± 294.1a	
15	1107.6 ± 121.2[a]	956.1 ± 128.8[a]
45	917.5 ± 185.3[a]	
80	838.3 ± 193.4[a]	
150	548.5 ± 131.5[a]	671.8 ± 181.2[a]
450	410.1 ± 44.4[a]	

[a] Significantly different form controls ($p < 0.05$)

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