Toxic equivalency factors derived from 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

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Introduction

Environmental exposure to polychlorinated dioxins (PCDDs), biphenyls (PCBs), and dibenzofurans (PCDFs) always involves complex mixtures of these compounds. Development of toxic equivalency factors (TEFs) for dioxin-like compounds has facilitated risk assessment of these mixtures. Each individual compound in that mixture is expressed as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) by using the relative potency to TCDD (Toxic Equivalency Factor or TEF) times its concentration. The summation of all the individual toxic equivalences equals the total dioxin-potency of that mixture[1]. This approach neglects possible interactive effects of the individual congeners.

Additive responses have been reported for mixtures of PCDDs (tumor promotion)[2] and mixtures of PCDDs and PCDFs (teratogenicity)[3]. Hepatic and pulmonary cytochrome P4501A1 (CYP1A1) induction after mixture exposure to PCDDs, PCDFs, and PCBs in female B6C3F1 mice in our laboratory was accurately predicted by using the TEF approach[4].

However, both antagonistic as well as synergistic effects between non-dioxin-like PCBs and TCDD following either acute or long-term exposure have been reported[1,5-7]. Non-dioxin-like PCBs, such as 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153), are quantitatively the most prominent polychlorinated biphenyl (PCB) congeners present in human tissue[8].

In this study, we tested if the TEF values derived from 90-day studies in female B6C3F1 mice can predict the response of a mixture containing PCDDs, PCDFs, and dioxin-like PCBs both in female B6C3F1 mice and Sprague Dawley rats. Furthermore, we tested if PCB 153 alters the response after TCDD exposure or after exposure to a mixture containing PCDDs, PCDFs, and dioxin-like PCBs in female B6C3F1 mice. The relative amounts of the individual congeners in the test mixture were selected to reflect those congeners found in food samples. The endpoints studied were CYP1A1 and CYP1A2 (liver only) activities in liver, lung, and skin (mice only).

Methods

Chemicals: TCDD, 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PCDF), 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), 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156), and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) 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 and female Sprague Dawley rats (both 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 and rats were randomly assigned to treatment groups (7 or 9 per group for the mice, 7 per group for the rats), and group housed. Animals were dosed by gavage with corn oil solutions of the test chemicals 5 days a week for 13 weeks. The mice were exposed to mixtures of 1: PCDDs, PCDFs, and PCBs (only planar and mono-ortho substituted PCBs) (1.0, 3.37, 33.7, and 337 ng TEQ/kg/day); 2: same mixture as 1 with the addition of the di-ortho substituted PCB 153 (1.0, 3.37, 33.7, and 337 ng TEQ/kg/day with 7.0, 23, 232, and 2323 µg PCB 153/kg/day; 3: PCB 153 alone (7.0, 23, 232, and 2323 µg PCB 153/kg/day); 4: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) alone (0.45, 1.5, 15, and 150 ng TCDD/kg/day); 5: TCDD (0.45, 1.5, 15, and 150 ng/kg/day) in co-administration with PCB 153 (7.0, 23, 232, and 2323 µg PCB 153/kg/day). The rats were exposed to A: TCDD alone (0.45, 1.5, 4.5, 15, 45, 80, 150, and 450 ng/kg/day); or B: the same mixture as 1 for the mice (1.0, 3.37, 33.7, and 337 ng TEQ/kg/day). The composition of mixture 1 (and B) is shown in Table 1. Three days after the last dose, animals were killed. Livers, lung, and skin were removed and S-9 fractions were prepared as described[9]. Microsomal fractions were prepared as described[9] and stored at -70°C until analyses.

Cytochrome P450 activities: Ethoxyresorufin *O*-deethylase activity (EROD), a marker for CYP1A1 activity, and methoxyresorufin *O*-demethylase activity (MROD), a marker for CYP1A2 activity, were determined as described before for EROD using 1.5 nM ethoxyresorufin or methoxyresorufin as substrates[9].

Statistics: Data were analyzed with one-way analysis of variance (ANOVA) and the least significant difference test (LSD; $p < 0.005$). Two-way analysis of variance was used to study interactive effects ($p < 0.05$).

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

Chemical	Food ratio	Relative potency values		
		TEF[7,10]	TEQ	Total % TEQ
TCDD	1.0	1	1	19
PCDD	1.0	0.7	0.7	14
TCDF	1.5	0.06	0.08	2
1PCDF	0.5	0.05	0.02	<1
4PCDF	2.0	0.25	0.5	10
OCDF	5.0	0.0003	0.001	<1
77	150	0.000005	0.0008	<1
126	45	0.055	2.5	48
169	30	0.001	0.03	1
105	6000	0.0000005	0.003	<1
118	30000	0.00001	0.3	5.8
156	1000	0.00001	0.01	<1

Results

Skin: Table 2 presents EROD activities in the skin after exposure to PCDDs, PCDFs, and PCBs in the mice. A dose-dependent induction of CYP1A1 activity was found in the skin after exposure to TCDD or the mixture of PCDDs, PCDFs, and the dioxin-like PCBs (TEQ-mix). The CYP1A1 activity for this mixture was the same range as for TCDD at similar dose levels: the TEQ-mix was about 4-fold less potent than expected. Co-administration of PCB 153 with TCDD alone or the TEQ-mix did not alter the TCDD or TEQ-mix induced CYP1A1 activity in the skin. PCB 153 alone did not alter CYP1A1 activities in the skin at all dose levels (data not shown).

Lung: Table 3 summarizes the CYP1A1 activities in the lung after exposure to the various mixtures in rats and mice. A dose-dependent induction of CYP1A1 activity was found in the lung after exposure to TCDD or the TEQ-mix in both mice and rats. The CYP1A1 activity for the TEQ-mix was the same as for TCDD at similar dose levels in the same species. The CYP1A1 activities after exposure to the same dose of TCDD or TEQ-mix were significantly higher in mice than in rats. Co-administration of PCB 153 with TCDD alone or the TEQ-mix resulted in significantly lower CYP1A1 activities in the mouse. PCB 153 alone did not alter CYP1A1 activities in the lung at all dose levels (data not shown).

Liver: Table 4 gives the CYP1A1 and CYP1A2 activities in the liver after exposure to the various mixtures in rats and mice. A dose-dependent increase in CYP1A1 and CYP1A2 activities was found in the liver after exposure to TCDD or the TEQ-mix in both the B6C3F1 mice and Sprague Dawley rats. Both CYP1A1 and CYP1A2 activities for the TEQ-mix were in the same range as for TCDD at similar dose levels: the TEQ-mix was about 4-fold more potent than expected in the mice and about 5-fold less potent than expected in the rats. Hepatic CYP1A1 activities after the same dose of TCDD were the same in rats and mice. The CYP1A2 activities after exposure to the same dose of TEQ-mix or TCDD were significantly higher in mice than in rats. Co-administration of PCB 153 with TCDD alone or the TEQ-mix resulted in significantly higher or lower CYP1A1 activities in the mouse, respectively. CYP1A2 activities in the mouse were the same or significantly lower after co-administration with PCB 153 and TCDD alone or the TEQ-mix, respectively. PCB 153 alone did not alter CYP1A1 and CYP1A2 activities in the liver at all dose levels (data not shown).

Discussion

Exposure to a mixture of PCDDs, PCDFs, and PCBs resulted in a dose-dependent CYP1A1 and CYP1A2 (liver only) induction in the liver, lung, and skin in female B6C3F1 mice and Sprague Dawley rats. The expected responses as calculated from the TEF values used (Table 1) were the same for TCDD alone in lung and within one order of magnitude in skin and liver. This indicates that the TEF-values used in this mixture to calculate the TEQ-dose accurately predict the effect in both female B6C3F1 mice and Sprague Dawley rats.

The species-difference in predicted response for hepatic tissue might be related to pharmacokinetic behavior of the administered compounds. Binary mixtures of dioxin-like compounds or TCDD and PCB 153 in female Sprague Dawley rats resulted in a decrease of the dioxin-like compounds in hepatic tissue[12,13]. In contrast, a binary mixture of TCDD and PCB 153 in female B6C3F1 mice did not result in alterations in hepatic concentrations of the administered compounds[14].

In mice, addition of PCB 153 to the TEQ-mix decreased the CYP1A1 and CYP1A2 (liver only) activities in the liver and lung, but was not altered in the skin. The decrease was again within one order of magnitude. This indicates that non-dioxin like PCBs modestly antagonize CYP1A1 and CYP1A2 induced activity by dioxin-like compounds in a tissue-specific manner.

[This abstract does not necessarily represent USEPA policy.]

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Table 2
EROD Activities in skin of Female B6C3F1 Mice after 13-week Exposure to Mixtures of PCDDs, PCDFs, and PCBs (mean ± SE).

Dose (ng TEQ/kg/day)	EROD mice (TEQ-mix) (pmol/min/mg)	EROD mice (TEQ-mix + PCB 153) (pmol/min/mg)
0	1.1 ± 0.1	1.2 ± 0.2
1.0	0.90 ± 0.21	0.82 ± 0.11
3.37	1.8 ± 0.6	1.5 ± 0.2
33.7	8.3 ± 1.0[a]	9.4 ± 1.0[a]
337	33.9 ± 1.3[a]	45.3 ± 3.5[a]
Dose (ng TCDD/kg/day)	**EROD mice (TCDD only) (pmol/min/mg)**	**EROD mice (TCDD + PCB 153) (pmol/min/mg)**
0	3.2 ± 0.7	3.4 ± 0.5
0.45	1.8 ± 0.2	2.0 ± 0.3
1.5	3.1 ± 0.6	1.6 ± 0.3
15	4.9 ± 0.3	6.1 ± 0.6
150	44.7 ± 13.2[a]	37.1 ± 3.2[a]

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

Table 3
EROD Activities in lung of Female B6C3F1 Mice and Sprague Dawley Rats after 13-week Exposure to Mixtures of PCDDs, PCDFs, and PCBs (*mean* ± SE).

Dose (ng TEQ/kg/day)	EROD rats (TEQ-mix) (pmol/min/mg)	EROD mice (TEQ-mix) (pmol/min/mg)[2]	EROD mice (TEQ-mix + PCB 153) (pmol/min/mg)[1]
0	0.2 ± 0.3	8.7 ± 3.0	12.3 ± 2.3
1.0	5.9 ± 1.5	7.3 ± 1.4	8.0 ± 1.4
3.37	5.8 ± 1.6	23.6 ± 3.9	15.1 ± 2.2
33.7	35.5 ± 4.2[a]	144 ± 4[a]	93.5 ± 8.0[a]
337	69.2 ± 9.3[a]	569 ± 54[a]	335 ± 43[a,b]
Dose (ng TCDD/kg/day)	**EROD rats (TCDD only) (pmol/min/mg)**	**EROD mice (TCDD only) (pmol/min/mg)[2]**	**EROD mice (TCDD + PCB 153) (pmol/min/mg)[1]**
0	1.1 ± 0.3	4.0 ± 0.6	2.9 ± 0.3
0.45	2.1 ± 0.5	3.4 ± 0.6	2.9 ± 0.3
1.5	2.7 ± 0.6	36.1 ± 4.4[a]	4.4 ± 0.6[b]
4.5	13.9 ± 2.5		
15	38.0 ± 5.8[a]	88.1 ± 12.8[a,c]	65.6 ± 4.8[a]
45	50.5 ± 10.9[a]		
80	58.2 ± 8.0[a]		
150	85.3 ± 17.6[a]	393 ± 33[a,c]	226 ± 12[a,b]
450	75.4 ± 7.1[a]		

1 Significant PCB effect was found ($p < 0.05$).
2 Significant species effect was found ($p < 0.05$).
a Significantly different from controls ($p < 0.05$).
b Significantly different from corresponding TEQ or TCDD dose alone ($p < 0.05$).
c Significantly different from corresponding dose in rats ($p < 0.05$).

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Table 4
Hepatic EROD and MROD Activities in Female B6C3F1 Mice and Sprague Dawley Rats after 13-week Exposure to Mixtures of PCDDs, PCDFs, and PCBs (mean ± SE).

Dose (ng TEQ/kg/day)	EROD rats (TEQ-mix) (pmol/min/mg)	EROD mice (TEQ-mix) (pmol/min/mg)[2]	EROD mice (TEQ-mix + PCB 153) (pmol/min/mg)[1]	MROD rats (TEQ-mix) (pmol/min/mg)	MROD mice (TEQ-mix) (pmol/min/mg)[2]	MROD mice (TEQ-mix + PCB 153) (pmol/min/mg)[1]
0	113 ± 10	463 ± 77	306 ± 11	27 ± 2	326 ± 61	288 ± 32
1.0	203 ± 12	392 ± 22	304 ± 17	45 ± 3	332 ± 54	278 ± 19
3.37	327 ± 36	419 ± 21	291 ± 15	67 ± 6	482 ± 38	315 ± 13
33.7	1434 ± 132[a]	3385 ± 312[a,c]	2280 ± 165[a,b]	205 ± 16	1752 ± 157	1203 ± 59[a,b]
337	2113 ± 82[a]	6968 ± 704[a,c]	3544 ± 110[a,b]	420 ± 23[a]	2831 ± 281	1908 ± 45[a,b]
Dose (ng TCDD/kg/day)	**EROD rats (TCDD only) (pmol/min/mg)**	**EROD mice (TCDD only) (pmol/min/mg)**	**EROD mice (TCDD + PCB 153) (pmol/min/mg)[1]**	**MROD rats (TCDD only) (pmol/min/mg)**	**MROD mice (TCDD only) (pmol/min/mg)[2]**	**MROD mice (TCDD + PCB 153) (pmol/min/mg)**
0	212 ± 38	233 ± 6	320 ± 19	37 ± 5	130 ± 5	253 ± 20
0.45	226 ± 20	299 ± 12	300 ± 49	63 ± 2	197 ± 13	252 ± 32
1.5	269 ± 16	211 ± 34	301 ± 19	85 ± 6	312 ± 47	302 ± 15
4.5	558 ± 64			144 ± 15		
15	1542 ± 70[a]	507 ± 47[a,c]	850 ± 69[a,b]	315 ± 12[a]	546 ± 49[a,c]	836 ± 40[a,b]
45	2404 ± 212[a]			395 ± 29[a]		
80	2564 ± 197[a]			431 ± 29[a]		
150	3838 ± 494[a]	3642 ± 210[a]	4295 ± 172[a,b]	490 ± 40[a]	1894 ± 189[a,c]	1687 ± 88[a,b]
450	3644 ± 377[a]			426 ± 22[a]		

1 Significant PCB effect was found ($p < 0.05$).
2 Significant species effect was found ($p < 0.05$).
a Significantly different from controls ($p < 0.05$).
b Significantly different from corresponding TEQ or TCDD dose alone ($p < 0.05$).
c Significantly different from corresponding dose in rats ($p < 0.05$).