Tissue Distribution of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and 2,2',4,4',5,5'-Hexachlorobiphenyl after Acute Oral Co-administration in Female B6C3F1 Mice

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Abstract

The distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) was studied in female B6C3F1 mice. TCDD alone (0, 0.1, 1, or 10 μg [^{3}H]TCDD/kg), PCB 153 alone (0, 3.58, 35.8, or 358 mg [^{14}C]PCB 153/kg), and all possible combinations of these doses were administered in corn oil, p.o. At 7 days after dosing, liver, fat, spleen, thymus, skin, lung, adrenals, kidneys, blood, muscle, and uterus were analyzed for radioactivity. TCDD-derived radioactivity distributed to all tissues in a dose-dependent manner, increasing with dose in the liver, while decreasing in all other organs. PCB 153 concentration was dose-dependently decreased in liver, skin, lung, adrenals, kidney, and blood; no dosimetric effects were observed in the other organs. Co-administration of low doses of both TCDD and PCB 153 gave little or no effect on the distribution of either compound. Interactive effects occurred on the pharmacokinetic behavior of both compounds only at higher doses. For example, the amount of TCDD in the liver was increased by PCB 153. In most other organs a PCB 153 dose-dependent decrease was found in the TCDD-content. Co-administration of PCB 153 with TCDD increased the hepatic PCB 153 distribution only. These results clearly demonstrate that interactive effects on pharmacokinetic behavior occur only at high doses.

Introduction

Exposure to polychlorinated dioxins (PCDDs), biphenyls (PCBs), and dibenzofurans (PCDFs) always involves complex mixtures of these compounds. Human risk assessment of exposure to these compounds is based on the concept of additivity of the individual dioxin-like congeners in a mixture. However, interactive effects between non-dioxin-like PCBs and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) following single-dose treatments have been reported. The non-additive interactive effects appear to depend upon the endpoint studied and the relative doses of TCDD and PCB administered. Short-term experiments in mice resulted in synergistic effects on hepatic microsomal monooxygenase activities after co-treatment with high doses of 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) and low doses of TCDD[1,2]. In rats, similar synergism was found following treatment of PCB 153 in co-administration with

3,3',4,4',5-pentachlorobiphenyl (PCB 126), 3,3',4,4',5,5'-hexachlorobiphenyl (PCB 169), or 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156)[3]. High doses of TCDD (near maximal enzyme induction) and high doses of PCB 153, do not result in synergistic interactions for hepatic microsomal monooxygenase activities, but may be slightly antagonistic[4]. Additionally, antagonistic effects on immunotoxicity and aryl hydrocarbon hydroxylase activity in mice were reported after co-administration of high doses of TCDD or Aroclor 1254 and PCB 153[5,6,7]. For the induction of cleft palate, again, antagonism was observed after co-administration of TCDD and PCB 153[4,8]. However, Morrissey and co-workers reported that renal abnormalities were enhanced in these animals[8].

Initial studies indicate that the antagonistic or synergistic effects may be due to alterations in the distribution of TCDD in the animal[2,4]. However, no extended dose-response relationships involving pharmacokinetic interactions have been conducted with these chemicals. In order to understand the interactions of TCDD with PCB 153, dose-response data are necessary for both TCDD and PCB 153. In this abstract the tissue distribution of both compounds are presented following single dosing and co-exposure. The effects on cytochromes P450 induction in various organs (liver, skin, and lung) and immunotoxicity are under investigation.

Methods

Chemicals: TCDD was purchased from Radian Corporation (Austin, TX) with purity >98% as determined by gas chromatography/mass spectrometry. [1,6-^{3}H]TCDD was synthesized by Chemsyn Science Laboratories (Lenexa, TX) with a specific activity of 34.7 Ci/mmole and purity of >98% as verified in our lab by HPLC before use. PCB 153 was obtained from Ultra Scientific (purity >98%). [^{14}C]PCB 153 was from Sigma Chemical Co. (St. Louis, MO) with a specific activity of 12.6 Ci/mole.

Animals and treatment: Female B6C3F1 mice (60 days old, ca. 20 g) were obtained from Charles River Breeding Laboratories, Raleigh, NC. Water and food were given *ad libitum*. The mice were held under controlled conditions of temperature (22°C ± 1) and lighting (12/12 light/dark cycle). Mice were randomly assigned to treatment groups (6 or 7 per group), and group housed. TCDD alone (0, 0.1, 1, or 10 μg [^{3}H]TCDD/kg), PCB 153 alone (0, 3.58, 35.8, or 358 mg [^{14}C]PCB 153/kg), and all possible combinations were administered in corn oil, p.o. At 7 days after dosing, liver, fat, spleen, thymus, skin, lung, adrenals, kidneys, blood, muscle, and uterus were analyzed for radioactivity.

PCB 153 and TCDD residue analyses: Residues of the administered compounds in all the tissues were analyzed for [^{3}H] and/or [^{14}C] activity by combustion (Packard 307 Sample Oxidizer Oximate 80, Downers Grove, IL) followed by liquid scintillation spectrometry (Packard, Downers Grove, IL).

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 ($p < 0.05$).

Results and Discussion

TCDD distribution: Table 1 presents the TCDD distribution in liver, adipose tissue and skin. Dose-dependent distribution of TCDD-derived radioactivity was demonstrated in all tissues. A significant increase in % dose per g or per tissue was found in the liver with increasing dosage of TCDD. Concentration (% dose per g) in adipose tissue decreased sublinearly as dose increased from 0.1 to 10 μg/kg. In all the other tissues a decrease in concentration (% dose per g tissue) was observed with increasing dosage. Similar dose-dependent kinetics have been found in mice and rats[9,10]. A possible explanation for the increase in hepatic TCDD distribution with increasing dose is the induction of a specific hepatic binding protein for planar polyhalogenated aromatic hydrocarbons, i.e., the CYP1A2 isozyme[11,12,13,14].
Co-administration of TCDD with PCB 153 showed little or no effect on TCDD distribution in all organs. The amount of TCDD in the liver was increased by PCB 153 only at higher doses of PCB 153. Again, this might result from an induction of the CYP1A2 binding protein by PCB 153 as demonstrated in mice by De Jongh and co-workers[2]. In most other organs a PCB 153-dependent decrease was found in the TCDD distribution.

PCB 153 distribution: Table 2 presents the PCB 153 distribution in liver, adipose tissue and skin. A PCB 153 dose-dependent decrease was found in liver, skin, lung, adrenals, kidney, and blood. No effect was observed in the other organs. This dose-dependent decrease in most organs suggests a dose-dependent decrease in gastrointestinal absorption of PCB 153. Co-administration of PCB 153 with the highest dose of TCDD influenced only the hepatic PCB 153 concentration (increase). Since PCB 153 has a higher affinity for adipose tissue than for liver (about 30-fold, see table 2), this increase in hepatic PCB 153 concentration might be due to an increase in its fat content by TCDD. For example, the planar 3,3',4,4'-tetrachlorobiphenyl was mainly located in the lipid droplets, mitochondria, and endoplasmatic reticulum, after a single dose in rats[15]. An increase in hepatic PCB 153 concentration after co-administration with TCDD was also found in female Sprague-Dawley rats after subchronic dosing[16].

In conclusion, the results of the present study demonstrate a dose-dependent tissue distribution for TCDD and PCB 153. Following acute exposure in the mouse, co-administration of PCB 153 and TCDD resulted in interactive effects on pharmacokinetic behavior only at high doses.

[This abstract does not necessarily represent USEPA policy.]

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Table 1

Distribution of TCDD-derived radioactivity after oral administration
Percent administered dose TCDD per gram tissue
(Percent administered dose TCDD per total tissue)

TCDD (μg/kg)	PCB 153 (mg/kg)	Liver % dose per g[1] (% dose per tissue)[1,2]	Adipose tissue % dose per g[1,2]	Skin % dose per g[1,2] (% dose per tissue)[1,2]
0.1	0	17.9 ± 0.7 (15.6 ± 0.7)	24.6 ± 1.6	3.38 ± 0.19 (5.97 ± 0.45)
1	0	26.6 ± 3.0[a] (26.7 ± 3.4)[a]	9.81 ± 0.61[a]	1.14 ± 0.08[a] (2.01 ± 0.20)[a]
10	0	33.3 ± 1.9[a] (37.6 ± 2.3)[a,b]	7.12 ± 0.59[a,b]	0.849 ± 0.061[a] (1.62 ± 0.15)[a]
0.1	3.58	18.8 ± 2.0 (17.0 ± 1.2)	22.5 ± 3.9	3.18 ± 0.32 (5.35 ± 0.61)
1	3.58	32.1 ± 2.5[f] (30.6 ± 1.6)[f]	11.0 ± 0.9[f]	1.60 ± 0.10[f] (2.83 ± 0.16)[f]
10	3.58	32.4 ± 1.5[f] (34.0 ± 1.4)[f]	7.81 ± 0.42[f]	1.14 ± 0.12[f] (2.10 ± 0.28)[f]
0.1	35.8	17.0 ± 1.7 (16.8 ± 1.4)	16.5 ± 1.1[a]	3.16 ± 0.45 (6.23 ± 1.08)
1	35.8	25.8 ± 3.0[f] (26.8 ± 3.1)[f]	9.84 ± 0.25[f]	1.36 ± 0.18[f] (2.63 ± 0.42)[f]
10	35.8	35.8 ± 3.4[f] (41.2 ± 5.2)[f,g]	7.14 ± 0.56[f]	0.804 ± 0.023[f,g] (1.30 ± 0.04)[f,g]
0.1	358	18.0 ± 0.8 (21.0 ± 1.0)	14.5 ± 0.7[a]	2.10 ± 0.19[a] (3.73 ± 0.37)[a,e]
1	358	26.3 ± 1.6[f] (37.6 ± 2.0)[b,e,f]	6.78 ± 0.27[b,d,e,f]	0.839 ± 0.086[d,e,f] (1.51 ± 0.14)[d,e,f]
10	358	32.7 ± 4.1[f] (47.7 ± 6.2)[f]	4.66 ± 0.21[c,d,e,f,g]	0.590 ± 0.028[c,d,f] (1.00 ± 0.05)[c,d,f]

Mean ± SE, n=6 or 7.
1 Significant TCDD effect was found ($p < 0.05$).
2 Significant PCB effect was found ($p < 0.05$).
a Significantly different from 0.1 μg TCDD/kg (LSD test, $p < 0.005$).
b Significantly different from 1 μg TCDD/kg (LSD test, $p < 0.005$).
c Significantly different from 10 μg TCDD/kg (LSD test, $p < 0.005$).
d Significantly different from corresponding TCDD dose in co-administration with 3.58 mg PCB 153/kg (LSD test, $p < 0.005$).
e Significantly different from corresponding TCDD dose in co-administration with 35.8 mg PCB 153/kg (LSD test, $p < 0.005$).
f Significantly different from corresponding PCB 153 dose in co-administration with 0.1 μg TCDD/kg (LSD test, $p < 0.005$).
g Significantly different from corresponding PCB 153 dose in co-administration with 1 μg TCDD/kg (LSD test, $p < 0.005$).

Table 2

Distribution of PCB 153-derived radioactivity after oral administration
Percent administered dose PCB 153 per gram tissue
(Percent administered dose PCB 153 per total tissue)

TCDD (μg/kg)	PCB 153 (mg/kg)	Liver % dose per g [1,2] (% dose per tissue) [1,2]	Adipose tissue % dose per g	Skin % dose per g [1] (% dose per tissue) [1]
0	3.58	1.37 ± 0.15 (1.26 ± 0.11)	42.3 ± 6.3	4.76 ± 0.44 (8.59 ± 0.90)
0.1	3.58	1.47 ± 0.12 (1.35 ± 0.08)	31.9 ± 7.7	5.10 ± 0.51 (8.54 ± 0.87)
1	3.58	1.42 ± 0.07 (1.37 ± 0.07)	33.4 ± 4.2	4.96 ± 0.23 (8.79 ± 0.32)
10	3.58	2.01 ± 0.18[a] (2.10 ± 0.16)[a,d,e]	31.9 ± 3.0	4.83 ± 0.30 (8.80 ± 0.82)
0	35.8	1.05 ± 0.14 (1.06 ± 0.13)	32.2 ± 5.4	4.05 ± 0.36 (8.15 ± 0.72)
0.1	35.8	1.00 ± 0.15[f] (0.991 ± 0.141)	27.4 ± 1.0	4.78 ± 0.69 (9.43 ± 1.61)
1	35.8	1.04 ± 0.10 (1.09 ± 0.10)	28.9 ± 2.0	4.19 ± 0.32 (8.02 ± 0.89)
10	35.8	1.66 ± 0.12[b,d,e] (1.91 ± 0.20)[b,d,e]	35.4 ± 3.5	4.07 ± 0.25 (6.58 ± 0.39)
0	358	0.872 ± 0.030[a] (1.06 ± 0.07)	29.1 ± 2.2	3.37 ± 0.25 (6.44 ± 0.54)
0.1	358	0.837 ± 0.039[f] (0.979 ± 0.059)	27.3 ± 1.8	3.79 ± 0.18 (6.71 ± 0.36)
1	358	0.879 ± 0.062[f] (1.27 ± 0.11)	30.0 ± 2.6	3.52 ± 0.42[f] (6.29 ± 0.71)
10	358	1.28 ± 0.18[f] (1.87 ± 0.27)[c,d]	28.7 ± 4.5	3.39 ± 0.29[f] (5.73 ± 0.40)[f]

Mean ± SE, n=6 or 7.
1 Significant PCB effect was found ($p < 0.05$).
2 Significant TCDD effect was found ($p < 0.05$).
a Significantly different from 3.58 mg PCB 153/kg (LSD test, $p < 0.005$).
b Significantly different from 35.8 mg PCB 153/kg (LSD test, $p < 0.005$).
c Significantly different from 358 mg PCB 153/kg (LSD test, $p < 0.005$).
d Significantly different from corresponding PCB 153 dose in co-administration with 0.1 μg TCDD/kg (LSD test, $p < 0.005$).
e Significantly different from corresponding PCB 153 dose in co-administration with 1 μg TCDD/kg (LSD test, $p < 0.005$).
f Significantly different from corresponding TCDD dose in co-administration with 3.58 mg PCB 153/kg (LSD test, $p < 0.005$).
g Significantly different from corresponding TCDD dose in co-administration with 35.8 mg PCB 153/kg (LSD test, $p < 0.005$).