Toxic Equivalency Factors for Mono- and Diortho-Substituted PCB Congeners in B6C3F1 Mice

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1. Introduction

Toxic equivalency factors (TEFs) have been extensively used for hazard and risk assessment of complex mixtures of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) [1]. TEFs for individual congeners represent their fractional potency relative to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Regulatory agencies have adopted TEF values for all 2,3,7,8-substituted PCDDs and PCDFs and they vary from 1.0 to 0.001 for TCDD and octachlorodibenzo-*p*-dioxin, respectively [2]. The toxic or TCDD equivalents (TEQs) of any mixture is defined as

$$TEQs = \sum [PCDD_i]\, TEF_i + \sum [PCDF_i]\, TEF_i$$

where $[PCDD_i]$ and $[PCDF_i]$ represent concentrations of individual congeners and TEF_i is their corresponding TEF. The coplanar or nonortho-substituted polychlorinated biphenyls (PCBs) and their monoortho-substituted analogs have also been characterized as aryl hydrocarbon (Ah) receptor agonists and a range of experimentally-derived TEFs have been determined [3]. This study reports the relative potency of several commercial PCB mixtures and mono- and diortho-substituted PCB congeners as inducers of hepatic microsomal ethoxyresorufin *O*-deethylase (EROD) activity in female B6C3F1 mice and their TEFs have been determined.

2. Materials and Methods

Chemicals. The Aroclors were generous gifts of Dr. B. J. Camp, formerly of Texas A&M University, and the individual PCB congeners were previously prepared in this laboratory [3].

Animal Treatment and Microsome Preparation. Three week old B6C3F1 female mice were received from an in house breeding colony and allowed to mature to 7 to 10 weeks of age before treatment. All animals were maintained on a 12 hour light/dark schedule with free access to food and water. Aroclors 1016, 1242, 1248, 1254, or 1260 and the PCB congeners were dissolved in corn oil and administered by i.p. injection in a total volume of 10 μL/g body weight.

Control animals received corn oil alone. Two days later, the mice were immunized with 50 μg of trinitrophenyl-lipopolysaccharide (TNP-LPS) in a total volume of 200 μL phosphate buffered saline (pH 7.4) by i.p. injection. Six days after the initial treatments, the mice were terminated by cervical dislocation. The liver was perfused with isotonic saline, removed, and placed in ice cold sucrose/EDTA solution. Tissue samples were homogenized, centrifuged at 10,000 *g* for 15 min at 4°C, and the supernatant centrifuged at 105,000 *g* for 1 hr to yield the microsomal pellet. The pellet was washed twice, resuspended in buffer, and diluted to 1 mg protein/ml. Suspensions were stored at -80°C until required for enzyme assays.

EROD Assay. EROD activities were measured by fluorimetric methods as previously described [4]. The production of resorufin was measured fluorimetrically with excitation and emission wavelengths of 550 and 585 nm, respectively. Protein concentrations were measured by the method of Bradford [5].

Statistical Analysis. An ED_{50} was estimated from the dose-response data for each test compound or mixture by means of a probit transformation. TCDD-induced hepatic microsomes were similarly used as a positive control for all EROD activities. Results are expressed as means ± SD for at least 4 animals for each treatment group.

3. Results and Discussion

The results presented in Table 1 summarize the dose-dependent induction of hepatic microsomal EROD activity by Aroclors 1260, 1254, 1248, 1242 and 1016. At the highest dose (1000 mg/kg), only Aroclor 1254 induced a maximal response corresponding to that observed for TCDD (8685 pmol/min/mg at the 3.6 μg/kg dose) and the order of potency for the Aroclors was Aroclor 1254 > 1242 > 1248 > 1260 > 1016. This order of potency differed from results of previous studies in Sprague-Dawley and Wistar rats in which either Aroclors 1242 or 1248 were more active as inducers [6,7]. The reasons for these interspecies differences in activity may be due to pharmacokinetic factors which influence hepatic levels and persistence of individual PCB congeners.

Table 1. Dose-dependent induction of hepatic microsomal EROD activity by commercial Aroclors in B6C3F1 mice.

	EROD Activity (pmol/min/mg)				
Mixture	0 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1000 mg/kg
Aroclor 1260	41 ± 3	215 ± 23	305 ± 27	602 ± 50	529 ± 66
Aroclor 1254	96 ± 8	294 ± 178	534 ± 265	3710 ± 429	8193 ± 2163
Aroclor 1242	101 ± 14	259 ± 54	408 ± 31	1305 ± 338	2386 ± 515
Aroclor 1016	75 ± 10	130 ± 14	164 ± 31	221 ± 35	276 ± 29
Aroclor 1248	29 ± 9	123 ± 33	246 ± 32	689 ± 140	950 ± 150

The results presented in Table 2 summarize the dose-dependent induction of hepatic microsomal EROD activity by mono- and diortho-substituted PCB congeners which are known to exhibit Ah receptor agonist activities and have previously been identified in commercial

Aroclors and environmental samples. With the exception of 2,2',3,4,4',5,5'-heptaCB which was essentially inactive, all congeners significantly induced EROD activity at the 25 mg/kg dose; however, at a dose of 150 or 200 mg/kg, none of the compounds induced maximal response (8685 pmol/min/mg) observed for TCDD (3.6 μg/kg).

Table 2. Dose-dependent induction of hepatic microsomal EROD activity by monoortho- and diortho-substituted PCB congeners.

	EROD Activity (pmol/min/mg)				
Congener	0 mg/kg	25 mg/kg	50 (75) mg/kg	100 mg/kg	150 (200) mg/kg
2,3,3',4,4',5,5'-heptaCB	147 ± 50	584 ± 120	1065 ± 105	1450 ± 270	4100 ± 75
2,3,3',4,4'-pentaCB	123 ± 11	979 ± 205	(838 ±78)	---	5820 ± 390
2,3,3',4,4',5-hexaCB	139 ± 14	601 ± 208	(3010 ± 710)	---	5575 ± 670
2,3',4,4',5-pentaCB	85 ± 23	175 ± 22	(297 ± 17)	---	826 ± 120
2,2',3,3',4,4',5-heptaCB	64 ± 7	---	223 ± 24	586 ± 127	(1330 ± 370)
2,2',3,4,4',5,5'-heptaCB	64 ± 7	---	106 ± 42	86 ± 7	(103 ± 12)

The induction-derived ED_{50} values were calculated for these congeners (Table 3) and their order of potency was 2,3,3',4,4',5-hexaCB > 2,3,3',4,4'-pentaCB > 2,3,3',4,4',5,5'-heptaCB > 2,2',3,3',4,4',5-heptaCB 2,3',4,4',5-pentaCB. In a parallel study, the ED_{50} value for TCDD was 0.37 μg/kg for induction of hepatic microsomal EROD activity in B6C3F1 mice (data not shown) and therefore TEF values were calculated for the PCBs used in this study (Table 3). The results show that induction-derived TEFs were significantly lower in B6C3F1 mice compared to female Sprague-Dawley (S-D) rats and they were also lower than the TEFs which have been proposed by a World Health Organization [8] committee. The data reported in this paper were obtained from animals used in an immunotoxicity study in which the immunotoxicity-derived TEFs for the same compounds varied from 3.1 to 5.5 x 10^{-5}. Thus, for both immunotoxicity and induction of hepatic EROD activity in B6C3F1 mice, the TEF values for the mono- and diortho-substituted congeners were lower than the WHO values. In contrast, the immunotoxicity-derived TEFs for the coplanar PCB congeners were significantly higher [9] than those recommended by the WHO committee [8]. These data further demonstrate that TEF values for PCB congeners are highly variable and dependent on a number of factors, including the response, target organ, animal species, duration and route of exposure. (Supported by the National Institutes of Health ES04917).

Table 3. Relative potencies and TEF values for PCB congeners in B6C3F1 mice.

		TEFs		
Congener	ED_{50} (mg/kg)	B6C3F1 Mice	S-D Rats[7]	WHO[8]
2,3,3',4,4',5-hexaCB	107	3.4 x 10^{-6}	---	.0001
2,3,3',4,4'-pentaCB	148	2.5 x 10^{-6}	1.25 x 10^{-4}	.0005
2,3,3',4,4',5,5'-heptaCB	299	1.2 x 10^{-6}	---	.0001
2,2',3,3',4,4',5-heptaCB	1125	3.0 x 10^{-7}	---	.0001
2,3',4,4',5-pentaCB	4785	7.7 x 10^{-8}	2.5 x 10^{-4}	.0001

5. References

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