Development and Validation of Toxic Equivalency Factors (TEFs) for PCBs in Female Sprague-Dawley Rats

K. Connor and S. Safe
Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77843-4466

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) have been widely detected as environmental contaminants and are also present in fish, wildlife, human adipose tissue, serum and breast milk. Background human exposure to these halogenated aromatic hydrocarbons (HAHs) occurs primarily via food intake. Environmental and food extracts invariably contain complex mixtures of HAHs and hazard assessment of these mixtures by various regulatory agencies has utilized the toxic equivalency factor (TEF) approach [1,2]. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic member of the HAHs and the TEF for individual PCDD and PCDF congeners has been defined as their fractional toxicity or potency relative to that of TCDD. Using this approach, the TEF values for the toxic 2,3,7,8-substituted PCDDs and PCDFs have been assigned and are used to determine dioxin or toxic equivalents (TEQs) of a mixtures of PCDDs and PCDFs where

$$TEQs = \sum [PCDD]_i TEF_i + \sum [PCDF]_i TEF_i$$

and TEF_i is the assigned TEF for each individual congener. The TEQs are routinely used for regulating emissions of PCDDs/PCDFs and assessing environmental levels and intakes of these compounds.

PCB mixtures also contain congeners which exhibit aryl hydrocarbon (Ah) receptor agonist activities and these include the coplanar chlorinated biphenyls (CBs) which are substituted in both para and a least two meta positions (3,4,4',5-tetraCB, 3,3',4,4'-tetraCB, 3,3',4,4',5-pentaCB and 3,3',4,4',5,5'-hexaCB) and their monoortho-substituted analogs [2-4]. TEF values have been assigned to these compounds and are utilized for calculating TEQs for PCB mixtures [4,5]. Several studies have reported that commercial PCBs and specific congeners typified by 2,2',4,4',5,5'-hexaCB inhibit several Ah receptor-mediated responses in the mouse including immunotoxicity and teratogenicity [6-9]. Moreover, the observed immunotoxicity of various commercial Aroclor mixtures in B6C3F1 mice is significantly lower than the calculated toxicity based on the TEF approach which utilized the concentrations of the congeners and their corresponding immunotoxicity-derived TEFs [9].

This study reports the dose-dependent induction of hepatic microsomal ethoxyresorufin O-deethylase (EROD) activity of several commercial PCBs and individual congeners in female Sprague-Dawley rats. The major long term goal of this research is to experimentally determine the role of non-additive (antagonist) interactions of PCB mixtures as inducers of EROD activity in the rat.

2. Materials and Methods

Animal Treatment and Microsome Preparation. Female Sprague-Dawley rats weighing 120 to 150 g were injected intraperitoneally with different PCB congeners or Aroclor mixtures in corn oil and, after 3 days, the animals were sacrificed by CO_2 asphyxiation and the livers perfused with isotonic saline and removed. 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 [10]. 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 [11].

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. Statistical differences were determined by the Student's t test.

3. Results

The results presented in Table 1 summarize the dose-dependent induction of hepatic microsomal EROD activity by Aroclors 1260, 1254, 1248, 1242 and 1016. Significant induction was observed at doses > 0.5 mg/kg for all the mixtures; based on ED_{50} values, the order of induction potency was Aroclor 1242 ~ Aroclor 1254 ~ Aroclor 1248 > Aroclor 1260 > Aroclor 1016.

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

	EROD Activity (pmol/min/mg)						
Mixture	0 mg/kg	0.5 mg/kg	5.0 mg/kg	50 mg/kg	150 mg/kg	500 mg/kg	ED_{50} (mg/kg)
Aroclor 1260	14 ± 3	16 ± 7.3	34 ± 13	149 ± 78	993 ± 218	2401 ± 555	83
Aroclor 1254	80 ± 16	93 ± 30	218 ± 177	4243 ± 524	4546 ± 393	4003 ± 595	17
Aroclor 1242	78 ± 11	104 ± 31	465 ± 545	2692 ± 1293	4253 ± 1636	4220 ± 1536	16
Aroclor 1016	82 ± 6.6	84 ± 7.3	76 ± 16	124 ± 20	268 ± 116	1081 ± 132	126
Aroclor 1248	62 ± 25	68 ± 4.2	500 ± 396	4445 ± 2080	7500 ± 861	7034 ± 1480	20.5

The induction-derived TEFs for 5 of the major monoortho and diortho coplanar PCBs present in commercial Aroclors and environmental mixtures have also been determined in female Sprague-Dawley rats (Table 2). Significant induction was observed at the 0.5 or 5.0 mg/kg doses and the order of induction potency for these compounds was 2,3',4,4',5-pentaCB > 2,3,3',4,4'-pentaCB ~ 2,3,3',4,4',5,5'-heptaCB > 2,2',3,3',4,4',5-heptaCB > 2,2',3,4,4',5,5'-heptaCB.

Table 2. Induction of hepatic microsomal EROD activity in female Sprague-Dawley rats by PCB congeners.

Congener	ED_{50} (mg/kg)	TEFs			
		This Study	Leece et al.	De Vito et al.	WHO
2,3',4,4',5-PentaCB	4	0.00025	0.0001	---	0.0001
2,3,3',4,4'-PentaCB	8	0.000125	< 0.000006	< 0.00005	0.0001
2,3,3',4,4',5,5'-HeptaCB	10	0.0001	0.00037	---	0.0001
2,2',3,3',4,4',5-HeptaCB	18	0.00006	---	---	0.0001
2,2',3,4,4',5,5'-HeptaCB	131	0.000008	---	---	0.00001

3. Discussion

All the Aroclor mixtures induced hepatic microsomal EROD activity and their order of potency was Aroclor 1242 ~ Aroclor 1254 ~ Aroclor 1248 > Aroclor 1260 > Aroclor 1016. Previous studies with Aroclors in male Wistar rats reported a somewhat different order of potency for these Aroclors in which Aroclor 1248 was the most potent inducer of EROD activity [12]. The differences between the present study and previous data [12] may be due to differences in duration of exposure to the Aroclors (3 vs 14 days) since the relative potencies of the more rapidly metabolized congeners are higher in the 3 day study (i.e. see Table 2 for TEF of 2,3,3',4,4'-pentaCB).

The results of preliminary studies with several major mono- and diortho coplanar PCB congeners are summarized in Table 2. Their ED_{50} values varied from 4 to 131 mg/kg and their corresponding TEFs were based on an estimated ED_{50} (EROD induction) for TCDD of 1 μg/kg. The TEF values obtained in this study were compared to the corresponding experimentally-derived TEF values determined in male Wistar rats treated with the same congeners for 2 weeks [13] (single dose, intraperitoneal) or B6C3F1 mice [14] for 4 weeks (oral gavage, 5 times/wk). The major difference between the various studies was observed for 2,3,3',4,4'-pentaCB which exhibited a higher TEF value in the short term (3 day) study. These results are not unexpected since this congener is readily metabolized and therefore lower tissue levels and EROD induction activity would be expected in longer term studies. For the remaining compounds, the experimentally-derived TEFs were similar for the 3, 14 and 28 day studies and these values exhibited considerable overlap with the corresponding TEF values proposed by the World Health Organization [5]. (Supported by the National Institutes of Health ES04917).

5. References

1. Bellin, J. S. and Barnes, D. G. (1989) *Interim Procedures for Estimating Risks Associated with Exposures to Mixtures of Chlorinated Dibenzo-p-Dioxins and -Dibenzofurans (CDDs and CDFs).* Washington, D.C. United States Environmental Protection Agency.
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). *C. R. C. Crit. Rev. Toxicol.* 21:51-88.
3. Safe, S. (1984) Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology and mechanism of action. *CRC Crit. Rev. Toxicol.* 12:319-395.
4. Safe, S. (1994) Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. *C. R. C. Crit. Rev. Toxicol.* 24:87-149.
5. Ahlborg, U. G., Becking, G. C., Birnbaum, L. S., Brouwer, A., Derks, H. J. G. M., Feeley, M., Golor, G., Hanberg, A., Larsen, J. C., Liem, A. K. D., Safe, S., Schlatter, C., Wærn, F., Younes, M., and Yrjänheikki, E. (1994) Toxic equivalency factors for dioxin-like PCBs. *Chemosphere* 28:1049-1067.
6. Biegel, L., Harris, M., Davis, D., Rosengren, R., Safe, L., and Safe, S. (1989) 2,2',4,4',5,5'-Hexachlorobiphenyl as a 2,3,7,8-tetrachlorodibenzo-*p*-dioxin antagonist in C57BL/6J mice. *Toxicol. Appl. Pharmacol.* 97:561-571.
7. Davis, D. and Safe, S. (1990) Immunosuppressive activities of polychlorinated biphenyls in C57BL/6 mice: structure-activity relationships as Ah receptor agonists and partial antagonists. *Toxicology* 63:97-111.
8. Haake, J. M., Safe, S., Mayura, K., and Phillips, T. D. (1987) Aroclor 1254 as an antagonist of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicol. Lett.* 38:299-306.
9. Harper, N., Connor, K., Steinberg, M., and Safe, S. (1995) Immunosuppressive activity of polychlorinated biphenyl mixtures and congeners: non-additive (antagonistic) interactions. *Fund. Appl. Toxicol.* (in press)
10. Pohl, R. J. and Fouts, J. R. (1980) A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. *Anal. Biochem.* 107:150-155.
11. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248-254.
12. Harris, M., Zacharewski, T., and Safe, S. (1993) Comparative potencies of Aroclors 1232, 1242, 1248, 1254 and 1260 in male Wistar rats - assessment of toxic equivalency factor (TEF) approach for polychlorinated biphenyls (PCBs). *Fund. Appl. Toxicol.* 20:456-463.
13. Leece, B., Denomme, M. A., Towner, R., and Li, S. M. A. (1985) Polychlorinated biphenyls: correlation between *in vivo* and *in vitro* quantitative structure-activity relationships (QSARs). *J. Toxicol. Environ. Health* 16:379-388.
14. 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 P4501A1 and 1A2 activity following weeks of treatment. *Fund. Appl. Toxicol.* 20:125-130.