TOXICITY EQUIVALENCY FACTORS FOR 'NON-DIOXIN-LIKE' PCBs - IN VITRO INDUCTION OF PROD ACTIVITY AS A SURROGATE?

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Introduction

Polychlorinated biphenyls (PCBs) represent a heterogeneous class of environmental contaminants. A few congeners lacking chlorine substituents at the ortho positions exhibit 'dioxin-like' properties in a variety of biochemical and toxicological test systems. Others bearing two or more chlorine substituents in *ortho*-positions virtually lack 'dioxin-like' properties more or less completely. These congeners are usually addressed as 'non-dioxin-like'. In between, a number of congeners with a single *ortho*-chlorine substituent seem to exert both 'dioxin-like' and non-dioxin-like' properties.
In 1998 an expert group of WHO[1] designated 12 PCBs as 'dioxin-like', the non-*ortho* substituted congeners #77, 81, 126, 169 and the mono-*ortho*-substituted congeners # 105, 114, 118, 123, 156, 157, 167 and 189. All of these congeners were attributed with Toxicity Equivalency Factors (TEFs) roughly reflecting their relative potency/toxicity in a number of test systems. This principle allows the calculation of a sum of TCDD or Toxicity Equivalents (TEq) giving an estimate for the total 'dioxin-like' toxicity of these congeners in a complex mixture. In most environmental samples , however, the amount of 'non-dioxin-like' PCBs exceeds that of the other congeners. Since a number of 'non-dioxin-like' PCBs have been shown or a likely to exert pronounced toxicity such as tumor promotion, neurotoxicity or teratogenicity/developmental toxicity in laboratory animals, the perspectives for a TEF system for those congeners has been discussed earlier.
In various *in vitro* systems relative potency factors (REPs) for aryl hydrocarbon receptor (AhR)-mediated effects such as induction of cytochrome P450 (CYP)1A isozymes have been derived for 'dioxin-like' PCBs. These factors are in good agreement with TEFs suggesting that most if not all of the toxic effects of these congeners are mediated via the AhR[2]. In contrast, no single *in vitro* parameter could be identified so far, to be used as a rough estimate for the relative *in vivo* potency(ies) of 'non-dioxin-like' PCBs.

Methods and Materials

Bovine serum albumin, collagenase type IV, and phenobarbital were from Sigma (Taufkirchen, Germany), Dulbecco's modified Eagle's medium (DMEM) from Seromed (Berlin, Germany), and Waymouth medium MD 705/1 from Gibco BRL (Heidelberg, Germany), ITS and ITS$^+$ from Becton Dickinson (Heidelberg, Germany) and the PCB congeners IUPAC number 28 (2,4,4'-trichlorobiphenyl), 101 (2,2',4,5,5'-pentachlorobiphenyl), 138 (2,2',3,4,4',5'-hexachloro-biphenyl), 183 (2,2',3,4,4',5',6-heptachlorobiphenyl), 187 (2,2',3,4',5,5',6-heptachlorobiphenyl), and 207 (2,2',3,3',4,4',5,6,6'-nonachlorobiphenyl) from Promochem (Wesel, Germany). All other chemicals were purchased at the highest purity commercially available. Male Wistar rats were obtained from Charles River (Kisslegg, Germany) and were kept under standard conditions. Adult animals at body weight of 150-180 g were anesthetized, and heopatocytes were prepared as described[3]. The cells were cultured using the collagen 'sandwich' procedure[4]. Hepatocytes were seeded at a density of 100,000 cells per cm^2 on collagen-coated 60 mm Petri dishes and were

incubated as described[3]. Twelve h after seeding PCBs dissolved in DMSO and phenobarbital dissolved in sterile saline were added. Controls were treated with DMSO or saline only. The cultures were washed, harvested, and homogenized 48 h after addition of the inducers. The 7-ethoxyresorufin O-deethylase (EROD) and 7-pentoxyresorufin-O-dealkylase (PROD) activities were analyzed using the method of Burke and Mayer[5].

Results and Discussion

All PCBs tested as well as phenobarbital led to a concentration-dependent induction of CYP 2B1/2B2-catalyzed 7-pentoxyresorufin O-dealkylase (PROD) activity in cell homogenates. Phenobarbital and the PCBs 101 and 187 exhibited a maximum efficacy in the range of 19 – 30 pmol/min x mg protein whereas treatment with PCB 28 (Fig. 1) resulted in a maximum level between 10 and 15 pmol/min x mg protein.

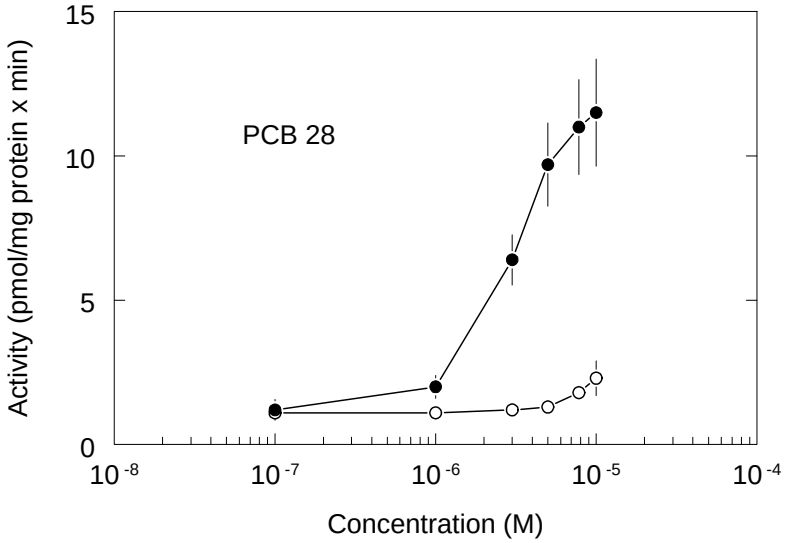
Table 1: Inducing potencies (EC_{50} values and 95% confidence intervals) of phenobarbital and a number of 'non-dioxin-like' PCBs as inducers of drug metabolism in rat hepatocytes in 'sandwich' primary culture

Inducer	**EC_{50} (M); EROD (pmol/min x mg protein)**	**EC_{50} (M); PROD (pmol/min x mg protein)**
Phenobarbital	**$1.8 \times 10^{-5} \pm 1.2 \times 10^{-5}$**	**$2.4 \times 10^{-5} \pm 0.2 \times 10^{-5}$**
PCB 28	**no induction**	**$3.3 \times 10^{-6} \pm 0.3 \times 10^{-6}$**
PCB 101	**no induction**	**$5.7 \times 10^{-6} \pm 1.0 \times 10^{-6}$**
PCB 138	**$3.5 \times 10^{-6} \pm 0.4 \times 10^{-6}$**	**$4.3 \times 10^{-7} \pm 0.4 \times 10^{-7}$**
PCB 153	**$1.2 \times 10^{-7} \pm 0.1 \times 10^{-7}$**	**$1.0 \times 10^{-7} \pm 0.2 \times 10^{-7}$**
PCB 183	**no maximum**	**$1.8 \times 10^{-7} \pm 1.2 \times 10^{-7}$**
PCB 187	**$1.2 \times 10^{-7} \pm 0.5 \times 10^{-7}$**	**$4.2 \times 10^{-7} \pm 0.3 \times 10^{-7}$**
PCB 207	**no maximum**	**$4.5 \times 10^{-7} \pm 0.4 \times 10^{-7}$**

With Phenobarbital, PCB 138 and 153 complete induction curves were obtained for the CYP1A-catalyzed 7-ethoxyresorufin O-deethylase (EROD) activity, whereas the PCBs 28 and 101 were inactive as EROD inducers. PCBs 183, 187, and 207 induced EROD activity to a limited extend without achieving a visible maximum (plateau) within the concentration range tested. Fitting of a sigmoidal concentration-response function to those experimental data showing a maximum/plateau, using a log-probit procedure, allowed the calculations of EC_{50}-values (95% confidence intervals; Table 1). With respect to PROD induction phenobarbital was about tenfold less potent than PCB 28. The inducing potencies of the PCBs as inducers of PROD followed the rank order 153 ≈ 183 >138 ≈187 ≈ 207 > 101 > 28.

A structural interpretation of these data appears difficult. Besides a tendency for higher PROD-inducing potency with an increasing number of chlorine substituents (from PCB 28 over PCB 101 to PCB 138), a high degree of chlorination led to a slight decrease in potency possibly related to a limited cellular availability (increasing lipophilicity) and/or to steric hindrance. EROD induction was independent of PROD induction as seen, e.g., with PCBs 28 and 101. The higher chlorinated PCBs 183 and 207 showed a flat EROD induction curve different from background at concentrations > 3×10^{-6}M (not shown) also indicating different mechanisms of induction

Fig. 1. Induction of EROD (- o -) and PROD (- • -) activities in rat hepatocytes in sandwich primary culture



Conclusions

Sandwich collagen primary culture of rat hepatocytes allows the induction of CYP2B1/2B2-catalyzed PROD. No striking structure-activity relationship for the PROD inducing potency of a number of 'non-dioxinlike' PCBs was observed. The findings indicate that the degree of chlorination and/or lipophilicity may play an critical role in PROD inducing potency. Thus PROD induction does not appear to provide a strong structure-discriminating indicator for toxicity comparable to AhR activation. Future studies are needed to clarify if any more differentiating relationship exists between chemical structure, PROD induction and the various toxic effects of this subclass of PCBs.

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