Antagonistic Effects of Mono- and Di-*Ortho* Substituted Polychlorinated Biphenyls in the Ethoxyresorufin-*O*-deethylase Activity and the Luciferase Reporter Gene Assay *in Vitro*

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Introduction

Polychlorinated biphenyls (PCBs), polychlorodibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and other polyhalogenated aromatic hydrocarbons (PHAHs) are present in the environment as complex mixtures. The toxic equivalency factor (TEF) concept has been developed for risk management purposes and allows to calculate the toxic potency of PHAH mixtures based on the assumptions that all dioxin-like congeners act through the same Ah receptor based mechanism and that the effects of individual compounds are additive[1,2]. However, in the past it has already been shown that some non-dioxin di-*ortho* substituted PCBs possess a low binding affinity for the Ah receptor[3,4] and interactions between congeners have been reported both *in vivo*[5,6,7,8] and *in vitro*[9]. Besides, Besselink *et al.*[10] reported an *in vitro* competitive inhibition of the CYP1A activity by several PCB congeners in rat liver microsomes.

The objective of this study was to determine whether mono- and di-*ortho* PCBs antagonize the AhR-mediated, 2,3,7,8,-TCDD induced ethoxyresorufin-*O*-deethylase (EROD) activity with different potencies and different maximum levels. To exclude the possibility of competitive inhibition of the CYP1A activity, combinations of congeners were also tested in the AhR mediated luciferase reporter gene (CALUX) assay.

Materials and Methods

Chemicals: 2,3,7,8-TCDD was obtained from Radian CIL, Inc. (USA) and PCBs were from Schmidt B.V. (Amsterdam, The Netherlands) and Ultra Scientific (North Kingstown, Ireland). All PHAH stock solutions were dissolved in dimethyl sulfoxide (DMSO 99.9%; Janssen Chimica, Geel, Belgium).

Exposures: Cells were exposed to single concentrations of mono- and di-*ortho* substituted PCB congeners alone and in combination with 50 pM 2,3,7,8-TCDD. Maximum DMSO

concentration in the medium was 0.1%. Mono-*ortho* PCBs tested with TCDD were: 2,3,3',4,4'-PeCB (PCB 105), 2,3,4,4',5-PeCB (PCB 114), 2,3',4,4',5-PeCB (PCB 118) and 2,3,3',4,4',5-PeCB (PCB 156). Di-*ortho* PCBs tested with TCDD were: 2,2',4,4'-TeCB (PCB 47), 2,2',5,5'-TeCB (PCB 52), 2,2',4,5,5'-PeCB (PCB 101), 2,2',3,3',4,4'-HxCB (PCB 128), 2,2',4,4',5,5'-HxCB (PCB 153) and 2,2',3,4,4',5,5'-HeCB (PCB 180). PCBs were tested in a concentration range of 0-66 μM. To calculate EC_{50} and IC_{50} values, a dose-response curve was fitted to the data using a the 1-site ligand binding equation $y=a_0x/(a_1+x)$.

EROD assay: EROD activity was measured using the mouse hepatoma Hepa1c1c7 cell line in 96-wells plates as described by de Haan *et al*[11].

CALUX assay: The CALUX-assay was performed in 96-wells plates as described by Murk *et al*[12] using a recombinant mouse hepatoma Hepa1c1c7 cell line carrying an AhR-responsive luciferase reporter gene.

Results and Discussion

All mono-*ortho* PCBs tested induced the EROD activity up to a plateau level of approximately 40-60% of the maximum TCDD induction, with EC_{50} values ranging between 1 and 33 μM (Table 1). The TCDD induced EROD activity was partially inhibited by the mono-*ortho* PCBs

Table 1 EC_{50} values of PCBs and the maximum measurable inhibiting effect on a 50 pM TCDD induced EROD and CALUX response

Congener	EROD			CALUX		
	EC_{50} (μM)	Maximum Inhibition (%)	IC_{50} (μM)	EC_{50} (μM)	Maximum Inhibition (%)	IC_{50} (μM)
PCB 105	3	49±8	1	6	61±8	1
PCB 114	2	36±8	4	6	40±12	13
PCB 118	2	26±5	1	n.a.	n. a.	n. a.
PCB 156	33	15±5	22	n.a.	n.a.	n. a.
PCB 47	n.d.	48±3	60	n.d.	66±1	40
PCB 52	n.d.	82±2	12	n.d.	87±1	10
PCB 101	n.d.	35±6	52	n.a.	n.a.	n.a.
PCB 128	n.d.	72±1	2	n.d.	80±1	2
PCB 153	n.d.	35±5	82	n.a.	n.a.	n.a.
PCB 180	n.d.	39±6	45	n.d.	14±3	-

n.d.=not detectable; n.a.=not analysed; Maximum inhibition is expressed as mean±standard deviation and defined as the inhibition measured at the highest tested concentration. IC_{50} is defined as the concentration inhibiting 50% of the EROD activity induced by 50 pM TCDD.

down to the maximum induction level of the PCB itself. This is clearly illustrated in figure 1, showing the dose-response curves for EROD induction by PCB 118 alone and in the presence of TCDD. The 'mirror'-like curve as shown for PCB 118 was observed for the other mono-*ortho* PCBs as well. No EROD induction was found for the tested di-*ortho* PCBs (Table 1). However, they all antagonized the TCDD-induced EROD activity in a dose-dependent manner and with different potencies (Figure 2, Table 1). IC_{50} values were calculated and given in table 1. The di-*ortho* substituted PCB 153 was the least potent inhibitor whereas the mono-*ortho* PCB 105 and 118 had the greatest inhibitory potency towards the TCDD induced EROD activity. The order with which the tested mono-*ortho* PCBs were capable of EROD inhibition reflects their potency to induce EROD activity. In the CALUX assay similar results were obtained (Table 1) for both the mono- and the di-*ortho* PCBs, indicating that substrate inhibition[10] doesn't play a role in the observed antagonistic effects. Although PCB 52 has no AhR-agonistic activity, Aarts *et al*[9] showed that PCB 52 (2,2',5,5'-TeCB) antagonized the

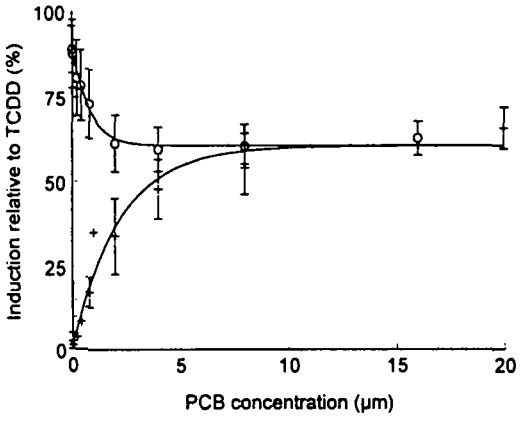

Figure 1 Inhibition of TCDD-induced (50 pM) EROD activity by increasing concentrations of PCB 118. Data are expressed as percentage induction relative to the induction of 2,3,7,8-TCDD at 1nM (plateau level). Data are given as the mean ± standard deviation.

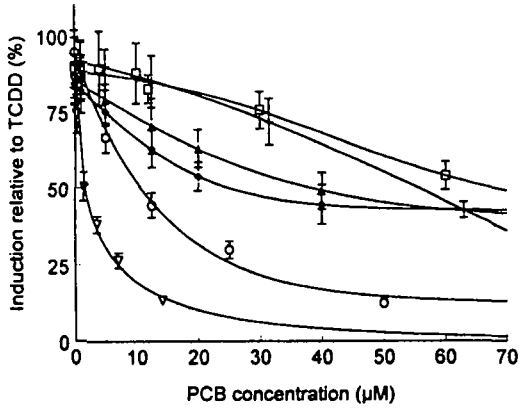

Figure 2 Inhibition of TCDD-induced (50 pM) EROD activity by increasing concentrations of di-*ortho* PCBs. Data are expressed as percentage induction relative to the induction of 2,3,7,8-TCDD at 1nM (plateau level). Data are given as the mean ± standard deviation.

2,2',3,3',4,4'-HxCB (PCB 77) induced luciferase expression and EROD activity *in vitro* by inhibition of Ah receptor ligand and DNA binding. This suggests that the observed antagonism in our study of both mono- and di-*ortho* PCBs on the TCDD induced EROD and CALUX activity is possibly explained by competition at the Ah receptor level.

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