Polybrominated Diphenyl Ethers (PBDEs) antagonize or inhibit TCDD induced CYP1A1 activity in various in vitro systems

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Introduction

Polybrominateddiphenylethers (PBDEs) are used as flame-retardant additive in a wide range of commercial products. Some PBDEs congeners are persistent in the environment, have lipophilic properties and consequently bioaccumulate. During the last decades, PBDE concentrations increased in fish, wildlife, and in humans[1-3]. In human milk some PBDEs doubled in concentration every 5 years since 1972[4] , but more recently this increase seems to have stopped and at least BDE47 is decreasing[5,6]. Structural similarity of certain PBDE congeners to other polyhalogenated aromatic hydrocarbons such as PCBs, has raised concerns that these could act as agonists for the Ah receptor (AhR)[7]. Recent studies in our laboratory have indicated that the most common PBDEs do not act as AhR agonists, measured as induction of CYP1A1 activity[8,9]. In this study we assessed the possible interaction between BDE47, 99, 100, 153, 154, and 183 and the AhR mediated CYP1A1 induction by TCDD in various in vitro models. BDE77 is not environmentally relevant, but resembles PCB77 was therefore also included in our experiments. These interactions were studied in AhR-containing human breast carcinoma (MCF-7), human hepatoma (HepG2), rat hepatoma (H4IIE) cells and primary hepatocytes from Cynomolgus monkeys. In addition, two stably transfected rodent hepatoma cell lines containing an AhR-responsive enhanced green fluorescent protein (EGFP)reporter gene, referred to as the chemically-activated fluorescence expression (CAFLUX) bioassay cells, were used to study AhR-dependent induction of EGFP and CYP1A1-associated enzyme activity. The mouse (H1G1.1c3) and rat hepatoma (H4G1.1c2) CAFLUX cell lines and their development has recently been described in detail[10,11].

Materials and Methods.

Chemicals. 2,3,7,8-TCDD (>99% pure) was obtained from Cambridge Isotope Laboratories (Woburn, MA, USA). PBDEs (>98% pure) were synthesized and subjected to purification on activated charcoal and Celite to remove possible contamination with dioxin-like compounds[12].

Cell lines and cell cultures. The MCF-7 and H4IIE cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and HepG2 cells from Deutsche Sammlung von Microorganismen und Zellkulturen (DSZM, Braunschweig, Germany). Culture conditions for the MCF-7, HepG2, H4IIE, and the CAFLUX cells (H1G1.1c3 and H4G1.1c2) have been described earlier[8,10,11] with some slight modifications that will be reported later.

Hepatocyte isolation from Cynomolgus monkey. Both male (n=3) and female (n=1) Cynomolgus monkeys (*Macacafascicularis*, 2-3 years old) were bred at the National Institute of Public Health and Environmental Protection (RIVM, Bilthoven, The Netherlands) and served as donors for kidney cells for production of poliomyelitis vaccine. This specific use did not have consequences for, or effects on the liver of these monkeys. Hepatocytes were isolated as described earlier[13].

Cell viability assays. Cell viability and cytotoxicity was tested using either the MTT, lactate dehydrogenase (LDH) or Alamar Blue assay[8].

EROD and protein assay. Ethoxyresorufin-O-deethylation (EROD) activity was used as a marker for CYP1A1

activity[14,15].Protein concentrations were determined using the method of Lowry[16].

Results and conclusions

MCF-7, HepG2, H4IIE cell lines.

A concentration-dependent decrease in TCDD-induced CYP1A1 (EROD) activity was observed when cells were co-incubated with PBDEs for 72h. While almost all PBDE congeners inhibited TCDD-dependent CYP1A1 induction, quantitative differences in the degree of inhibition were apparent. This difference in potency is illustrated for the combination of TCDD and BDE 153 in the MCF-7 cells and significant inhibition could be observed in the concentration range 1 to 10 µM BDE153 (fig. 1).Comparable results were obtained with HEPG2 and H4IIE cells.

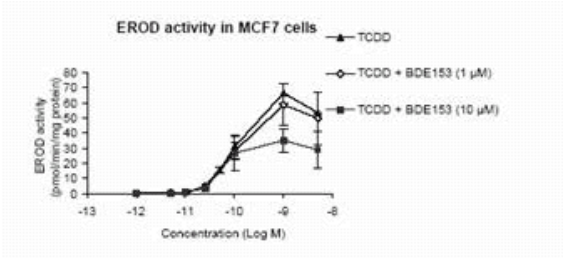


Figure 1. Inhibition by BDE 153 (1, 10 µM) on TCDD-mediated induction of CYP1A1 activity in MCF-7 cells. Data expressed as mean ± standard deviation (n=3) (From Peters et al., 2004).

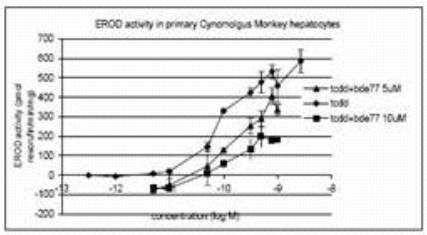
The reduction in TCDD-induced CYP1A1 activity was not caused by cytotoxicity and direct exposure experiments during the EROD assay did not indicate direct catalytic inhibition of the CYP1A1 activity. These results have been recently reported in detail[8]. BDE-209 was excluded as the responsible inhibitory compound since it was insoluble under our experimental conditions.

Cynomolgus monkey hepatocytes.

Similar results were obtained for the inhibition of TCDD induced CYP1A1 activity in monkey hepatocytes for all environmentally relevant PBDEs. As an example the interaction between BDEs 77 and 153 on TCDD CYP1A1 induction is shown in fig. 2 a en b.

Hepa1c.1c7 and H4G1.1c2 (CAFLUX) cell lines.

In both CAFLUX cell lines environmentally relevant PBDEs and BDE77 showed a significant decrease in TCDD-induced CYP1A1 (EROD) activity. For lower brominatedPDBEs like BDE 47, this decrease mirrored the decrease in EGFP reporter gene expression (see fig 3). The latter suggests that observed antagonism or inhibition of TCDD induced CYP1A1 might be a direct result of interaction of these PBDEs with the AhR. However, it was also observed that some of the higher brominatedPBDEs like BDE 183 could reduce TCDD induced CYP1A1 activity, but did not affect the level of EGFP gene expression. This observation suggests that more than one mechanism could be responsible for the observed PBDE inhibitory effects.



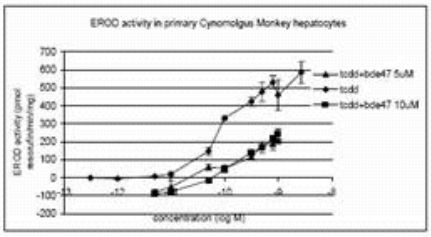
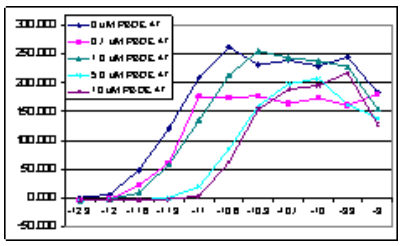


Figure 2 A and B. Inhibition by BDE 47 and 77 (5, 10 µM) on TCDD-mediated induction of CYP1A1 activity in hepatocytes of Cynomolgus monkeys. Data expressed as mean ± standard deviation (n=3).



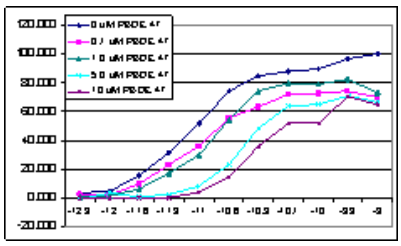


Figure 3 A and B. Inhibition by BDE 47 (0.1 to 10 µM) on TCDD-mediated induction of fluorescence (CAFLUX) and CYP1A1 (EROD) activity in the H4G1.1c2 cell line.

Our results indicate that PBDEs are able to interfere with AhR mediated processes like TCDD-induced CYP1A1 activity in rodent and human cell lines as well as in primary primate hepatocytes. The combined results indicate that this effect might be due to interactions of the AhR with lower brominatedPBDEs. However, effects on TCDD-induced CYP1A1 activity for some of the higher brominatedPBDEs may be AhR independent. This mechanism should be investigated further. Based on these results, the question is to which extent these PBDEs could actually inhibit or antagonize AhR mediated toxicological and biological effects. The occurrence of such an effect in the *in vivo* situation would primarily depend on the internal exposure of both dioxin like compounds and PBDEs, the relative ratio at which both compounds occur *in vivo*, their relative AhR binding affinity, inducing potency of the dioxin like chemicals and the inhibitory potency of the individual inhibitory PBDE.

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