IN VITRO ANTI-ANDROGENICITY OF PBDES, HBCD, TBP AND HYDROXYLATED AND METHOXYLATED PBDES BASED ON A YEAST BIOASSAY

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Abstract
Polybrominated diphenylethers (PBDEs), hexabromocyclododecane (HBCD) and tribromophenol (TBP), used as flame retardants (FR), have been detected in environmental as well as biological samples, e.g. mammalian tissues and fluids. PBDEs are susceptible to several in vivo metabolic processes including oxidative and reductive debromination, oxidative CYP enzyme-mediated biotransformation, and/or phase II conjugation. Hydroxy- and methoxylated PBDEs (OH-PBDEs, CH_3O-PBDEs) have recently been reported to be present in various biotic samples including wildlife and humans. Some *in vitro* studies indicate that OH-PBDEs are potential endocrine disruptors (e.g. OH-PBDEs and brominated bisphenol A-analogs are agonists of the estrogen receptor) .
During the last two years, our laboratory focused on environmentally relevant BFRs, including some PBDE metabolites, and their possible effects on sex hormone synthesis and *in vitro* metabolism. TBP induced CYP19 significantly activity and many OH-PBDEs decreased CYP17 and CYP19 activities at lower µM concentrations. In this study, the androgen receptor (AR) agonist and antagonist potencies of several brominated FR and PBDE metabolites were studied in a yeast bioassay. Our results show that several OH-BDEs have antagonistic properties on the AR receptor at in vitro concentrations below 1 µM.

Introduction
Brominated flame retardants (BFRs) are chemicals used in all kind of materials for electronic and daily used apparatuses, to reduce fire risks. These compounds act in the gas phase of the fire by reacting with free radicals generated during combustion, thus terminating the reaction [1]. From an environmental point of view, due to the intensified use of e.g. electronic equipment, BFRs have become an increasingly important group of organohalogen compounds, which include among others polybrominated diphenylethers (PBDEs), tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBCD) isomers [2]. The global production of PBDEs only was about 40,000 tons in 2001 [3]. PBDEs can be divided into three major commercial mixtures, penta-, octa- and deca-BDEs. The first two PBDE mixtures have been recently banned in Europe and in North America, through voluntary actions by industry. PBDEs are now found commonly in the abiotic and biotic environment; many of these are lipophilic and persistent, thus having the potential to bioaccumulate and/or biomagnify [4]. Since the mid 1970s to 1980s, a substantial increase in PBDE concentrations, for example in mother's milk, was observed [5]. However, a slight decrease during the last 5 years in several environmental samples has been observed in North Europe [6], most likely due to the ban of the penta- and octa-BDE mixtures. PBDE metabolites, mainly tetrabromo- and pentabromo-methoxylated (CH_3O-PBDEs) and hydroxylated (OH-PBDEs) PBDEs, have also been detected in blood and to a lesser extent in adipose and liver tissues, in some fish, bird, and mammalian species [7, 8]. In addition, some of these PBDE metabolites have been detected in human blood [9]. Furthermore, a number of OH-PBDEs have been isolated and structurally identified as natural compounds in marine sponges and ascidians (tunicates) [10, 11]. All natural occurring OH-PBDEs in these marine organisms have a hydroxyl group at the ortho position relative to the ether bound, and are exemplified by 6-OH-BDE47 and 2'-OH-BDE68 [12]. *In vitro* studies have shown that some OH-PBDEs can bind competitively to the thyroid hormone transport protein transthyretin (TTR) [13] and are estrogenic [14]. Using the human adrenocortical carcinoma cell line (H295R), some PBDE derivatives also inhibit CYP19 (aromatase) and CYP17 activity, key enzymes in steroidogenesis, significantly; although to these inhibitory effects could partly be attributed to cytotoxicity [15, 16].Using an AR-CALUX reporter gen system, *in vitro* anti-androgenic effects have

been shown previously for some of these BFRs, especially PBDEs. Interestingly, in some cases the potency of individual congeners was higher than the natural ligands [17]. In order to study further the possible effects of BFRs and PBDE metabolites on the androgen receptor (AR) in more detail, a new highly specific yeast androgen bioassay was used [18, 19]. In these experiments BFRs and reference compounds were tested on potential agonistic as well as their antagonistic potencies.

Material and Methods

Chemicals. In this study, the AR recombinant yeasts cells were exposed to a selection of BFRs i.e., tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCD), 2,4,6-tribromophenol (TBP), and a number of PBDEs or their hydroxy or methoxy derivatives (Table 1). The compounds were synthesized at the Wallenberg Laboratory (Stockholm University, Sweden). Stock solutions were 2.5mM, and different dilutions (from 0.01 up to 25 μM) were used to test.

AR yeast androgen bioassay The yeast biosensor, expressing the human androgen receptor (hAR) and yeast enhanced green fluorescent protein (γEGFP) in response to androgens, was developed at the Institute of Food Safety (RIKILT), Wageningen, The Netherlands [18]. Antagonistic properties of test compounds were examined by co-exposure to a concentration of testosterone (T2, 1μM) that induces half of the maximum response.

Data analysis. Each sample concentration was tested in triplicate. Graphs, statistical significant differences among means (one-way ANOVA) and IC_{50} calculations were done using Prism 3.0 (GraphaPad Software Inc. San Diego, CA, USA).

Results and Discussion

Recombinant yeast cells that express the human androgen receptor (AR) and yeast enhanced green fluorescent protein (γEGFP) were used to assess the possible AR agonistic and antagonistic potency of a group of 40 selected BFRs, including PBDE and some of their metabolites.

All compounds were tested at concentrations ranging from 0.01 up to 25 μM. The specificity of the AR yeast bioassay was demonstrated with exposures to flutamide, a positive control for androgen antagonism, 17β-Estradiol (estrogen receptor agonist) and dexamethasone (glucocorticoid receptor agonist), were used as negative controls in the AR yeast assay.

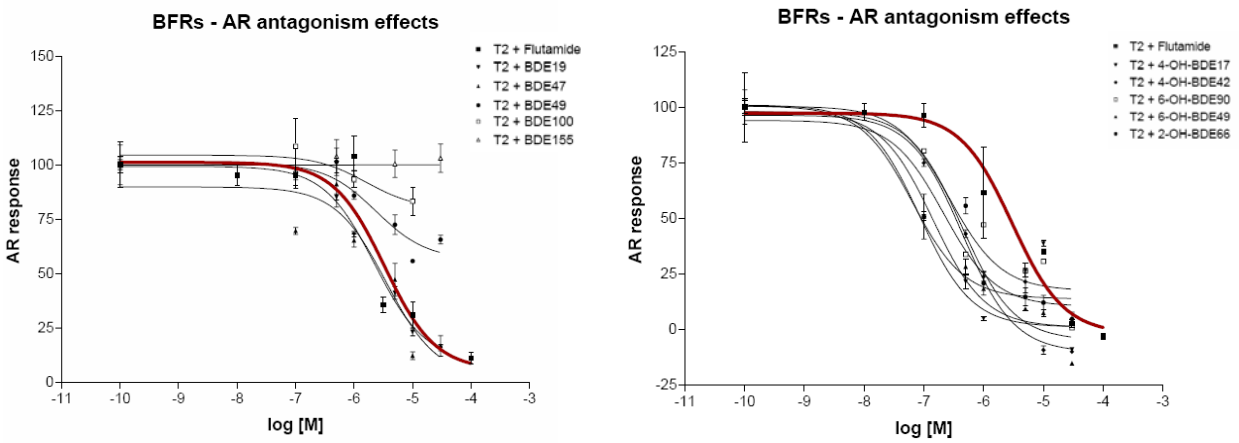
Interestingly, none of the tested BFRs or their metabolites showed agonistic potencies in the AR yeast assay. However, 19 out of 40 BFRs and PBDE derivatives showed anti-androgenic effects when co-exposed with T2, one of the most potent endogenous AR ligands, at a concentration that caused a half a maximum response (EC_{50}) in this assay (1μM). Flutamide completely decreased T2-induced fluorescence with an IC_{50} value of 1 μM. Significant AR antagonism was found for BDE19, 47, 49 and TBP, which caused a concentration-dependent, decrease of the T2-induced response with more than 80% at 25 μM (see Figure 1A).

Exposures to the OH-PBDEs also resulted in a concentration-dependent anti-androgenicity in most cases, with a reduction of a T2-induced response between 50 and 90% at 25 μM (see Figure 1B). Some of the CH_3O-PBDEs also displayed anti-androgenic activity, but not more than 50% of T2-induced response, except for 2-CH_3O-BDE28 showing a decreased of almost 90% at the highest concentration tested and an IC_{50} value of 8,9 μM. Previous *in vitro* experiments using the AR-CALUX methodology, showed similar anti-androgenic properties of these compounds, but in some cases with higher potencies compared to our results [14, 17].

The combined results of these studies show that some of the BFRs and especially some PBDEs metabolites exhibit anti-androgenic properties that can already be observed around 1 μM or lower. For some of these PBDE metabolites an AR antagonistic potency was observed that was similar or even higher than flutamide, a selective AR antagonist (see Figure 1B).

Further experiments are needed to determine if possible cytotoxic effects of these test compounds to the AR yeast or AR CAFLUX cells can (partly) explained the observed AR antagonism.

Figure 1A **1B**



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