DETERMINATION OF THE ENDOCRINE DISRUPTING POTENCY OF HYDROXYLATED PCBS AND FLAME RETARDANTS WITH IN VITRO BIOASSAYS

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Introduction

Organohalogenated compounds (OHS) are one of the most prominent and persistent classes of environmental pollutants that has been associated with adverse health effects in humans and wildlife. Recent evidence has shown that PCB levels in human blood plasma have not changed between the beginning of the 1990s and 1999 (1), suggesting that the decline in PCBs in food and the environment may have leveled off. There are also several new classes of OHS that are still in use as high production volume chemicals in Europe and the rest of the world, such as the brominated flame retardants, polybrominated bisphenols and –diphenylethers (BDEs). These compounds have recently been shown to incline in the environment (2). Moreover, they show a striking resemblance in structure, environmental fate and toxicological effects with "old" OHS classes (1, 3). Recent studies have identified a large number of hydroxylated metabolites of these classes of organohalogens in human blood (4), which apparently are formed in considerable amounts *in vivo*. Within the E.U. project "Comparison of Exposure-Effect Pathways to Improve the Assessment of Human Health Risks of Complex Environmental Mixtures of Organohalogens" (COMPARE), comparative pathways for early life-stage exposure and long-term effects of hydroxylated PCBs and PBDEs are under investigation. The *in vitro* endocrine disrupting potency of a number of hydroxylated OHS was studied using CALUX® reporter gene assays based on cell lines stably transfected with dioxin-receptor (DR) and estrogen receptor (ER)-mediated luciferase gene constructs. In addition, the potency of the compounds to interfere with thyroid hormone (T4) transport via binding to the transport protein transthyretin (TTR) was measured with an *in vitro* T4-TTR competitive binding assay.

Methods and Materials

Test compounds including the hydroxylated PCB metabolites 4-OH-CB 107 and 4-OH-CB 187, PBDE 47 and its hydroxylated metabolite 6-OH-BDE 47, tetrabromobisphenoal A (TBBPA) and metabolite 2,4,6 tribromophenol (TBP), were dissolved in DMSO. The *in vitro* Estrogen-Responsive Chemical Activated LUciferase gene eXpression (ER-CALUX®) assay, which measures luciferase expression in stably transfected human T47D breast cancer cells expressing endogenous estrogen receptors, was performed as described previously (5). The dioxin-responsive DR-CALUX® assay uses rat hepatoma cells expressing endogenous Ah receptor and was performed as described previously (6). In both assays, cells are seeded in 96 well plates, incubated with test compounds for 24 hours and luciferase protein is assayed by lysing the cells, adding luciferin substrate, and measuring light photon production. The T4-TTR competitive binding assay, which measures the ability of the test compound to interfere with the natural hormone T4 binding to the plasma transport protein TTR, was measured as

described previously (3). Briefly, test compounds are incubated for 24 hours in a test tube containing ^{125}I labeled T4, non-radioactive T4 and purified human TTR. Test compounds bound to TTR (thereby displacing T4 from the protein) are separated from unbound compounds over a gel column. Radioactivity in the column eluate is measured and indicates the TTR binding capacity of the test compound.

Results and Discussion

In vitro dioxin-like and estrogenic effects of hydroxylated OHS

The potency of hydroxylated OHS to bind to and transactivate estrogen and dioxin receptors was investigated using the ER- and DR-CALUX® assays. In the ER-CALUX® assay, BDE 47 was the only compound which showed estrogenic activity, albeit by concentrations exceeding 1000 nM (Figure 1, Table 1). Of the compounds tested for dioxin-like activity, only 6-OH-BDE induced luciferase activity in the DR-CALUX® assay, with a potency of about 2000 times less than TCDD (Figure 1, Table 1). The PCB metabolite 4-OH-PCB 107 showed antagonistic effects on both the ER and DR-CALUX® responses (Table 1). BDE 47 (5 mM) demonstrated anti-dioxin like activity in the DR-CALUX® assay.

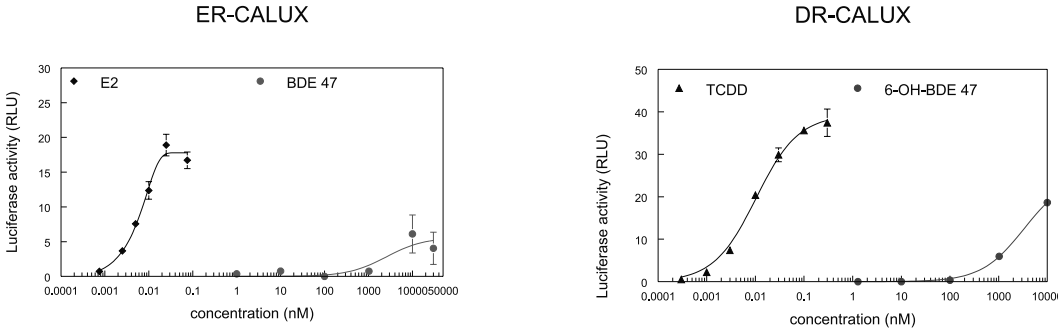

Figure 1. Estrogenic activity of BDE 47 in the ER-CALUX® and dioxin-like activity of 6-HO-BDE 47 in the DR-CALUX® assay.

In vitro thryoidogenic effects of hydroxylated OHS

In the TTR-binding competition assay, all compounds tested, with the exception of BDE 47, showed displacement of radioactive labeled thyroxin (T4) and binding to the thyroid hormone transport protein TTR (Table 1). The hydroxylated PCBs were the most potent inhibitors of T4 binding to TTR (Table 1). While 4-OH-CB 107 has also been shown previously to bind TTR (7), this is the first demonstration that 4-OH-CB 187 is just as potent in displacing T4 from TTR (up to 10x more potent than T4, Figure 2). The flame retardant TBBPA and metabolite 2,4,6 tribromophenol, were also more potent relative to T4 in binding TTR (Table 1). The hydroxylated BDE 6-OH-BDE 47 was about 20 times less potent than T4.

The disruption of thyroid hormone transport observed *in vitro* with OH-PCBs and OH-BDEs will be further investigated *in vivo* by studying the comparative long-term developmental, reproductive and behavioural effects of early life-stage exposure to these compounds. This risk assessment approach is essential as previous studies have shown extensive placental transport of hydroxylated PCBs resulting in relatively high fetal blood plasma levels of hydroxylated OHS in both experimental animals (7) and

Table 1. Endocrine disrupting potency of organohalogens and hydroxylated metabolites in the ER-, DR-CALUX® and T4-TTR competitive binding assays. Antagonistic activity was measured by combining test compounds (5 mM) with 6 pM estradiol (ER-CALUXÒ) or 10 pM TCDD (DR-CALUX®) and is expressed relative to estradiol or TCDD alone.

test compound	Estrogenic activity (EC25 nM)	Anti-estrogenic activity	Dioxin-like activity	Anti-dioxin activity (IC50 nM)	T4-TTR binding
TCDD	-	-	0.03	-	-
Estradiol	0.003	-	-	-	-
Thyroxin	-	-	-	-	80
4-OH-PCB 107	0	69%	0	87%	14
4-OH-PCB 187	0	0	0	0	15
BDE 47	2200	0	0	52%	0
6-OH-BDE 47	0	21%	10000	0	270
TBBPA	0	0	0	0	35
2,4,6 TBP	0	0	0	0	40

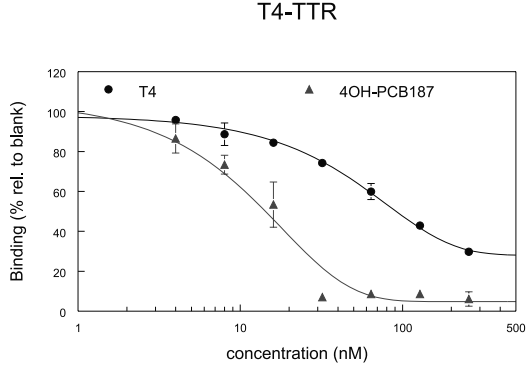

Figure 2. Displacement of thyroxin (T4) from transthyretin (TTR) by 4-OH-CB 187.

in human infants (1). Prenatal exposure to low levels of 4-OH-CB 107 has resulted in adverse effects on the estrous cycle length, uterine effects and altered estrogen levels as well as altered behavioural development and dramatically lowered thyroid hormone levels in rat offspring (7). In addition to extensive *in vivo* studies, research will be undertaken to further validate the *in vitro* assays for measurement of total organohalogen exposure as rapid and sensitive biomarkers of exposure in biomedical and epidemiological studies.

Acknowedgements

This study has been financed by the European Union, contract no. QLRT-2000-00261.

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