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Are brominated flame retardants endocrine disruptors?

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Abstract

Brominated flame retardants (BFRs) are a group of compounds that have received much attention recently due to their similarity with "old" classes of organohalogenated compounds such as polychlorinated biphenyls (PCBs), in terms of their fate, stability in the environment and accumulation in humans and wildlife. Toxic effects, including teratogenicity, carcinogenicity and neurotoxicity, have been observed for some BFR congeners, in particular the brominated diphenyl ethers (BDEs). This concise review focuses on the potency of BFRs and to disrupt endocrine systems, and attempts to answer the question whether or not BFRs are endocrine disruptors. Evidence is provided on the disruption of the thyroid hormone system by BFRs, with particular emphasis on the BDEs, as most recent data is available on this class of flame retardants. Similar to the hydroxylated PCBs, in vitro mechanistic studies as well as animal experiments have demonstrated the effects of BDEs on thyroid hormone transport and metabolism. An overview of possible effects of BFRs on the estrogen system is also provided. Research gaps are outlined, as well as ongoing and future studies in the European community aimed at contributing to comprehensive risk assessments based on the endocrine-disrupting effects of BFRs.

Keywords: Flame retardants; Endocrine; Polychlorinated biphenyls

1. Introduction

Organohalogenated compounds are some of the most prominent and persistent classes of environmental pollutants associated with adverse health effects in humans and wildlife. Recent studies in Sweden have shown that polychlorinated biphenol (PCB) levels in human blood plasma have not changed between the beginning of the 1990s and 1999 (Bergman et al., 1999), suggesting that the decline in PCBs in food and the environment may have levelled off. There are also several new classes of organohalogens still in use as high production volume chemicals in Europe and abroad, including the brominated flame retardants (BFRs) such as brominated bisphenols and brominated diphenylethers (BDEs). These "new" classes of compounds show a striking resemblance in structure to "old" classes such as PCBs. Levels of BDEs have recently been shown to increase in various compartments in the environment (Bergman, 2000; Ikonomou et al., 2002). Hydroxylated metabolites of BDEs have also been identified in human samples, including blood (Hovander et al., 2002) and breast milk (Meironyté et al., 1999).

In addition to the similarity between structure, environmental fate and levels of "old" and "new" classes of organohalogens, these compounds have been shown to share some of the same mechanisms of action mediating their toxicity, in particular on endocrine systems. Comprehensive reviews on the toxicology and potential health risks of the flame retardant BDEs have been recently written, including their demonstrated teratogenic, carcinogenic, genotoxic, endocrine and neurotoxic effects (Darnerud et al., 2001; McDonald, 2002). The objective of this paper is to concisely summarize recent studies on the endocrine-disrupting potency of BFRs. An endocrine-disrupting compound has been defined as "an exogenous agent which interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body which are responsible for the maintenance of homeostasis, reproduction, development or behaviour" (Kavlock et al., 1996). This wide definition includes all substances that can affect endocrine function via interference with hormone (e.g. estrogen, androgen, or thyroid hormone) pathways. Using the "old" classes of (hydroxylated) organohalogens such as PCBs as a comparison, this review focuses in particular on the potency of BFRs to interfere with thyroid hormone and estrogen endocrine systems. We provide clear evidence that BFRs are potential endocrine disruptors of

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these hormonal pathways, as demonstrated in both in vitro and in vivo test systems. Research gaps are outlined, and an overview of ongoing BFR studies in the European community is also provided.

2. Interactions of BFRs with the thyroid hormone system

Several studies have shown that BFRs, in particular BDEs, share the general property of organohalogenated compounds in which in vivo exposure in rodents results in reduction of serum total and free thyroid hormone (thyroxine (T4)) levels (Fowles et al., 1994; Zhou et al., 2001, 2002; Hallgren et al., 2001; Hallgren and Darnerud, 2002). The mechanism of action of interference of organohalogens with the thyroid hormone system may involve three levels of interaction: (a) thyroid gland function and regulation; (b) thyroid hormone metabolism; and (c) thyroid hormone transport mechanisms (Brouwer et al., 1998). Evidence of direct effects of BFRs on *thyroid gland function* is limited. Mice chronically exposed to deca-BDE in feeding studies have demonstrated an increased incidence of thyroid hyperplasia and tumors (NTP, 1986). Thyroid hyperplasia was also observed in rats orally exposed for 90 days to high concentrations (100 mg/kg) of DE-71, a mixture of tetra-, penta- and hexa-BDEs, in the diet (WIL, 1984). The BFR 2,2-bis(bromomethyl)-1,3-propanediol (BMP) has been shown to induce neoplasms in the thyroid gland in rats exposed through the diet (Dunnick et al., 1997). In human epidemiological studies, occupational mixed exposure to deca-BDE and polybrominated biphenyls has been associated with increased prevalence of primary hypothyroidism (Bahn et al., 1980).

There are a number of pathways of *thyroid hormone metabolism*, including sulfation, deiodiation and glucuronidation (reviewed in Visser, 1996), through which organohalogens may exert their toxicity. One of the effects of hydroxylated PCBs on thyroid hormone metabolism demonstrated in vitro is the inhibition of thyroid hormone sulfation (Schuur et al., 1998), which is a major regulation pathway of free hormone levels in the fetus. Similarly, the BFR tetrabromobisphenol A (TBBPA) has also been shown to inhibit thyroid hormone sulfation in vitro at the same low micromolar range of other halogenated organochlorine compounds (Schuur et al., 1998). Hydroxylated organohalogens have also been shown to affect T4 metabolism through inhibition of deiodinase activity, thereby preventing the formation of the active thyroid hormone 3,3′,5-triiodothyronine (T3) (Lans, 1995). However, to our knowledge, no effects of BFRs on deiodination have been reported until now. In contrast, the induction of liver glucuronidase activity by BFRs, which is related to increased elimination of T4, has been reported in a number of studies. Short-term (4 day) oral administration of 0.3–300 mg/kg/day DE-71 (penta- and tetra-BDE mixture) and DE-79 (octa- and hepta-BDE mixture) in weanling rats resulted in dose-dependent depletion of T4, which was accompanied by up to four-fold induction in uridinephospho-glucuronyltransferase (UDPGT) activity (Zhou et al., 2001). Developmental exposure to DE-71 also showed elevated UDGPT activity in offspring of rats exposed to 1–30 mg/kg/day from gestation day 6 to postnatal day 21 (Zhou et al., 2002). Exposure of female rats to 18 mg/kg/day to the pure tetra-BDE congener (BDE-47) for 14 days by gastric intubation led to moderate elevation of UDPGT activity (Hallgren and Darnerud, 2002). Oral 18 mg/kg/day exposure of rats and mice to the commercial PBDE mixture Bromkal 70-5 DE or the tetra-BDE BDE-47 for 14 days lead to only slight UDPGT induction, though free and total T4 levels were significantly reduced (Hallgren et al., 2001).

Disruption of *thyroid hormone transport* by organohalogens has been revealed in a number of studies, in which decreased levels of circulating plasma T4 have been reported following (hydroxylated) PCB exposure in both adult and developing rats (Brouwer and van den Berg, 1986; Morse

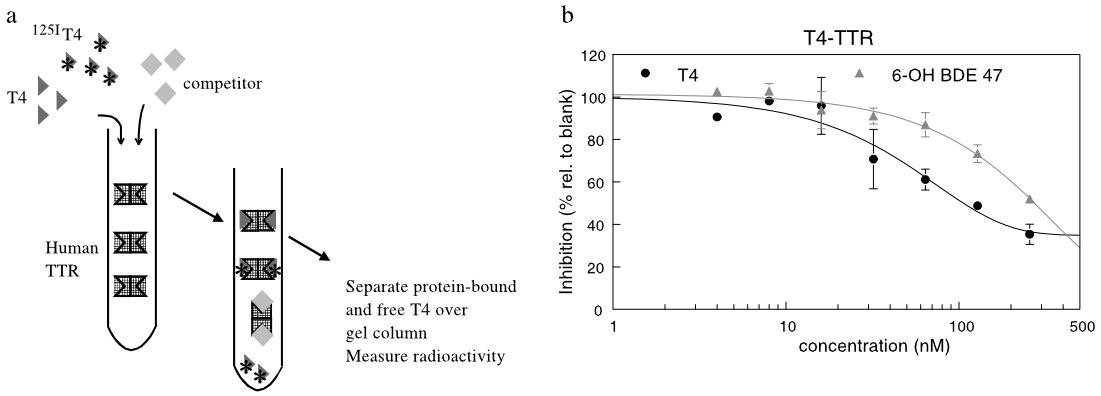

Fig. 1. (a) Principle of thyroxine (T4)–transthyretin (TTR) competitive binding assay in which T4, radioactively labelled ^{125I}T4, and competitors are incubated with purified human TTR; and (b) inhibition of TTR–^{125I}T4 binding by T4 and the hydroxylated plasma metabolite 6-OH-BDE 47 (adapted from Legler et al., 2002).

and Brouwer, 1993; Morse et al., 1996a; Meerts, 2001). Hydroxylated metabolites of PCBs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans have been shown to bind with high affinity to the plasma thyroid hormone transport protein transthyretin (TTR), thereby displacing the natural ligand T4 (Lans et al., 1993; Morse et al., 1996a). TTR is synthesized in liver and brain, and is important for maternal to fetal transport of thyroid hormones in vertebrates, as well as for delivery of T4 across the blood–brain barrier (Schreiber et al., 1995). The in vitro T4–TTR competition-binding assay can be used to rapidly demonstrate the affinity of xenobiotic compounds for binding to TTR. As shown in Fig. 1a, increasing concentrations of potential competitors are mixed with purified human TTR, and a fixed amount of ^{125}I-labelled T4 and unlabelled T4. Following overnight incubation, protein-bound and free ^{125}I-T4 are separated on a biogel column and radioactivity is determined in the eluate containing protein-bound ^{125}I-T4 fraction. Comparison of T4 standard concentration curves with competitor concentration curves (as shown in Fig. 1b) yields IC_{50} values, from which potencies of compounds relative to T4 are calculated (Table 1). Previous studies have shown that some BFRs are very potent competitors for T4 binding to TTR in vitro (Meerts et al., 2000; Table 1). Importantly, TBBPA, and the brominated plasma metabolite 2,4,6-tribromophenol show binding to TTR with higher affinity than T4 itself (up to 10 times, Table 1). A recent study has also shown that TBBPA and tetrachlorobisphenol A (TCBPA) inhibit binding of triiodothyronine (T3) to thyroid hormone receptors, as well as stimulate growth of TH-dependent rat pituitary GH3 cells (Kitamura et al., 2002). BDEs appear only able to compete with T4 for binding to TTR after metabolic conversion to (hydroxylated) metabolites, as shown when parent compounds were first metabolized using phenobarbital-induced (mainly CYP2B) liver microsomes (Meerts et al., 2000; Table 1). Recent studies in our laboratories have shown that the metabolite 6-OH-BDE 47 found in vivo in human plasma (Bergman et al., 1999; Hovander et al., 2002) binds to TTR with relatively high affinity (Fig. 1b, Table 1). Structure–activity relationships indicate that the degree of bromination, the nature of the halogen substitution (i.e. bromine or chlorine) and the substitution pattern of the bromine atoms played crucial roles in the binding potency of compounds to bind to TTR (Meerts, 2001; Ghosh et al., 2000).

The high in vitro affinity of some hydroxylated organohalogenated compounds to TTR has been confirmed for hydroxylated-PCBs in in vivo studies with rodents, in both fetal and maternal compartments (Darnerud et al., 1996; Meerts et al., 2002). Similarly for the BFRs, ex vivo binding of BDE-47 to plasma TTR has been shown following oral exposure of female rats (Hallgren and Darnerud, 2002), confirming that (metabolites of) BFRs are transported in vivo by TTR. The binding of hydroxylated organohalogens to TTR in vivo may result in facilitated transport over the placenta to the fetal compartment, leading to decreased thyroid levels in both the mother and foetus with consequences for foetal brain development, as was shown following prenatal hydroxylated-PCB exposure (Morse et al., 1996a,b). Indeed, Meerts et al. (2002) confirmed that the important human plasma PCB metabolite 4-OH-CB 107, when administered in utero, accumulates in the foetal compartment, resulting in significantly decreased levels of foetal plasma T4. Prenatal exposure to 4-OH-CB 107 also resulted in several subtle behavioural effects in offspring, including impaired locomotor activity and effects on passive avoidance (catalepsy) (Meerts, 2001). Accordingly, other studies have shown that neonatal exposure of rodents to the BFRs BDE-47 and BDE-99 (Eriksson et al., 2001; Viberg et al., 2002; Eriksson et al., 2002a) as well as BDE-153 and hexabromocyclododecane (Eriksson et al., 2002b) can cause developmental neurotoxic effects. In these studies, neuro-

Table 1
In vitro endocrine disrupting potency of some brominated flame retardants

Compound	Thyroid hormone-like activity		Estrogenic activity	
	Binding to transthyretin		Luciferase induction ER-CALUX	
	Relative potency[a]	Reference	Relative potency[b]	Reference
Thyroxine	1		–	
Estradiol	–		1	
BDE 15	n.d.[c]	1	n.d.	3
BDE 28	n.d.[c]	1	2×10^{-6}	3
BDE 30	n.d.[c]	1	2×10^{-6}	3
BDE 32	n.d.	1	2×10^{-5}	3
BDE 47	n.d.[c]	1, 2	2×10^{-7}	2, 3
BDE 51	n.d.[c]	1	2×10^{-6}	3
BDE 71	n.d.	1	2×10^{-6}	3
BDE 75	n.d.[c]	1	2×10^{-6}	3
BDE 77	n.d.[c]	1	n.d.	3
BDE 85	n.d.	1	2×10^{-7}	3
BDE 99	n.d.	1	2×10^{-7}	3
BDE 100	n.d.[c]	1	2×10^{-5}	3
BDE 119	n.d.[c]	1	2×10^{-5}	3
BDE 138	n.d.	1	$>>10^{-7}$	3
BDE 153	n.d.	1	n.d.[d]	3
BDE 166	n.d.	1	n.d.[d]	3
BDE 190	n.d.	1	n.d.[d]	3
6-OH-BDE 47	0.3	2	n.d.	2
Monobromobisphenol A	n.d.	1	1×10^{-5}	3
Dibromobisphenol A	n.d.	1	1×10^{-5}	3
Tribromobisphenol A	0.6	1	1×10^{-6}	3
Tetrabromobisphenol A	10	1, 2	1×10^{-6}[e]	4
2,4,6-Tribromophenol	2	1, 2	n.d.	2, 3

n.d.: not detected.
References: (1) Meerts et al. (2000); (2) Legler et al. (2002); (3) Meerts et al. (2001); (4) Korner et al. (1998).
[a] Potency relative to thyroxine calculated as ratio IC_{50} T4/IC_{50} compound.
[b] Potency relative to estradiol calculated as ratio LOEC E2/LOEC compound.
[c] Binding to TTR observed in metabolites following pre-incubation of parent compounds with phenobarbital-induced liver microsomes.
[d] Compound anti-estrogenic when co-administered with estradiol.
[e] Estrogenic activity calculated using cell proliferation assay (E-screen).

toxic effects were related to changes in the cholinergic system, an important transmitter system involved in cognitive function. In the studies with 4-OH-CB 107, behavioural effects were related to the low levels of T4 (Meerts et al., 2002). While thyroid hormone levels were not reported in the BFR neurotoxicity studies, other studies have shown that BDE-47 and BDE-99 exposure results in decreased T4 levels in offspring (see above). The implications of altered thyroid hormone homeostasis are profound, and have been hypothesized to lead to disrupted brain development and permanent neurological damage (reviewed in Darnerud et al., 2001; Morreale de Escobar et al., 2000).

3. Interactions of BFRs with the estrogen system

Though the evidence of the thyroidogenic properties of BFRs far outweighs that of their estrogenic potency, a number of studies have indicated that these compounds may interfere with estrogen pathways. Firstly, some BFRs have been shown to bind to and activate estrogen receptors. A recently developed in vitro estrogen receptor-mediated reporter gene assay using stably transfected human breast cancer cells (ER-CALUX assay) has been used to determine the (anti-)estrogenic potency of a number of PBDEs, hydroxylated PBDEs and brominated bisphenols. In the ER-CALUX assay, an ER-mediated luciferase reporter gene construct containing three estrogen response elements (EREs) has been stably introduced and integrated in the genome of the T47D cells (Legler et al., 1999). Exposure of cells to (xeno-)estrogens results in diffusion of chemicals through the cell membrane, binding to the endogenous ER, activation of the receptor, and consequently, binding of the ligand–receptor complex to EREs present in the promoter region of the luciferase gene. Luciferase protein is then induced, and is easily measured by lysing the cells, adding luciferin substrate, and measuring light photon production (Fig. 2a). In experiments with BFR exposure in the ER-CALUX assay, BDE-47 showed weak estrogenic activity in the ER-CALUX assay compared to estradiol (Fig. 2b). Other BDE congeners found to possess estrogenic activity include BDE-100, BDE-75 and BDE-51, with potencies of about 10^{-6} relative to estradiol (Meerts et al., 2001; Table 1). Interestingly, some polybrominated bisphenol (PBBP) analogs (e.g. mono- and dibromobisphenol A) showed higher estrogenic potency than the BDEs (Table 1). In other studies, the PBBP TBBPA also showed binding to ERs as well as inducing proliferation of estrogen-dependent MCF-7 cells (Korner et al., 1998; Samuelsen et al., 2001) and MtT/E2 cells (Kitamura et al., 2002). Some BDEs also show anti-estrogenic potency when tested in vitro at micromolar concentrations in combination with estradiol, including BDE-153, BDE-166 and BDE-190 (Meerts et al., 2001). It should be mentioned that a recent study failed to reveal the estrogenic potency of 10 BDEs including BDE-47, BDE-100 and BDE-75 (Villeneuve et al., 2002). This discrepancy may be due to the higher (10-fold) sensitivity to estrogens found in the ER-CALUX as compared to the MVLN reporter gene assay (Legler et al., 1999).

Studies on the specific estrogenic effects of BFRs in vivo are limited. Despite its in vitro estrogenic activity, studies with in ovo exposure of TBBPA have revealed no estrogenic effects in quail and chicken embryo development (Berg et al., 2001). This apparent lack of estrogenicity may be due to the rapid metabolism of TBBPA in vivo, as shown in both quail (Halldin et al., 2001) and rats (Meerts et al., 1999). Future studies (see below) may elucidate if the striking effects of hydroxylated PCBs on the estrogen system previously observed in vivo, i.e. prolonged estrous cycles and elevated plasma estradiol concentrations in prenatally exposed female offspring (Meerts, 2001), are also observed with BFR exposure.

4. Research gaps and ongoing research in Europe

It is clear from the studies summarized above that some BFRs may be considered potential endocrine disruptors.

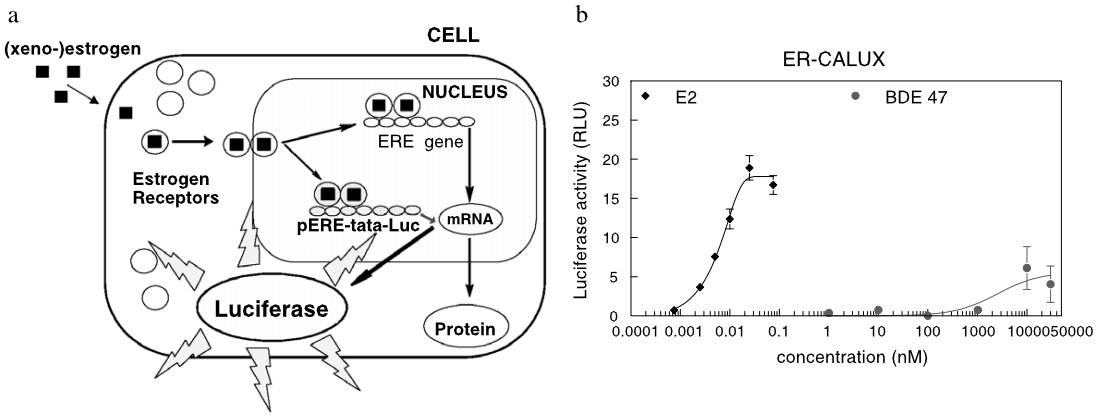

Fig. 2. (a) Principle of estrogen action in the ER-CALUX assay with stably transfected T47D breast cancer cells, Legler et al., 1999; and (b) dose-related induction of luciferase activity by estradiol (E2) and brominated diphenyl ether BDE 47 (adapted from Legler et al., 2002).

The effects of BDEs demonstrated in rodent studies on thyroid hormone homeostasis are particularly relevant. In humans, adverse health outcomes such as neurodevelopmental toxicity, goiter and thyroid diseases are associated with thyroid hormone disruption (Morreale de Escobar et al., 2000). Pregnant females and developing embryos and infants are especially responsive to small changes in thyroid hormone homeostasis, and should be viewed as sensitive populations for exposure to BDEs (McDonald, 2002). However, the toxicological profile of many BFRs is too incomplete and insufficient to perform an adequate human and ecological risk assessment. From an endocrine disruption viewpoint, additional mechanistic and toxicological studies of thyroid function are needed for the most prominent BFR congeners and metabolites found in (human) tissues and the environment. As effects on neurodevelopment may be related to altered thyroid hormone status, studies focussing on developmental toxicity and neurobehavioural changes are essential. In addition to effects on thyroid hormone systems, there is some mechanistic evidence for the effects of BFRs on other hormonal systems, such as the estrogen system (see above). However, at this time, information is too scarce to assess the effects of BFRs on hormone-related endpoints and reproduction.

Currently, two large multi-partner European Union projects are underway to address some of these research gaps. The first project is entitled "Comparison of Exposure-Effect Pathways to Improve the Assessment of Human Health Risks of Complex Environmental Mixtures of Organohalogens" (COMPARE; www.compare-project.info). The main objective of the COMPARE project is to investigate comparative pathways for early life-stage exposure and long-term effects of organohalogens including PCBs and the flame retardants polybrominated bisphenols and BDEs and their hydroxylated metabolites. Ultimately, this research aims to provide a mechanism-based approach for the assessment of human health risks from exposure to organohalogens. Some specific aims of the project include:

- identification, characterization and synthesis of different classes of organohalogens, including BFRs, and their major metabolites present in human blood plasma and food;
- comparison of the kinetics and in particular the maternal to fetal transfer of (hydroxylated) organohalogens in laboratory rodents;
- interspecies comparison (rodents, birds, human) and chemical specificity of hormone binding proteins (e.g. TTR) as a facilitated transplacental transport system for hydroxylated organohalogens;
- comparison of long-term adverse developmental (reproductive and behavioural) effects of pre-natal exposure to some representative congeners of different classes of organohalogens;
- epidemiological studies of possible organohalogen exposure–health effect relationships in adult human individuals (osteoporosis/endometriosis) and infants (neurobehavioural impact of early (fetal) exposure).

Model test compounds to determine long-term adverse developmental effects have been selected based on their presence in human plasma and the environment, as well as their high volume use in Europe. Due to their prevalence in human plasma, the hydroxylated PCB metabolites 4-OH-CB 107 and 4-OH-CB 187, as well as 6-OH-BDE 47 and 2,4,6-tribromophenol, have been selected as test compounds. BDE 47 has been included as a test compound due to its high levels in the environment and biota, whereas TBBPA was selected as a model high-volume production BFR. The toxicological effects of these compounds are currently being investigated in detailed rodent in vivo studies focusing on long-term developmental, reproductive and behavioural effects of prenatal exposure to these compounds.

While the COMPARE project aims to compare toxicological profiles of "old" (PCB) and "new" (BFR) classes of organohalogens, a second research project has recently started in which the main focus is on the endocrine-disrupting potency of BFRs. The European Union funded project "Risk Assessment of Brominated Flame Retardants (BFRs) as Suspected Endocrine Disrupters for Human and Wildlife Health" (FIRE), coordinated by the Dutch National Institute for Environmental Health (RIVM), aims to identify and characterize the presence of the major BFRs (including BDE congeners, TBBPA and hexabromocyclododecane) in abiotic and biotic samples from the European environment. The endocrine-related effects of major BFRs will be determined using in vitro assays as pre-screening methods for in vivo studies with BFRs. In vitro studies will include (anti-)estrogenic, (anti-)androgenic and (anti-)thyroid hormone mediated responses, effects on sex hormone synthesis and metabolism, and effects mediated by the progesterone receptor and the Ah receptor. In vivo studies with BFRs will involve two-generation reproduction experiments with rodents, as well as partial life cycle studies in fish species including flounder and zebrafish. More information on this project can be found at http://www.rivm.nl/fire/.

In conclusion, substantial evidence exists indicating that BFRs are potential endocrine disruptors. In particular, their potency to disrupt thyroid hormone mediated pathways has been demonstrated in both in vitro and in vivo studies, in which they have been shown to bind to thyroid hormone transport proteins and evoke reductions in thyroid hormone levels in offspring of exposed animals. Future studies, including the EU-funded projects described above, are crucial to fill in the research gaps necessary for an integrated risk assessment of these compounds for both human and ecosystem health.

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