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ORIGINAL ARTICLE

Brominated flame retardants as possible endocrine disrupters

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Summary

Brominated flame retardants (BFR) are endocrine disrupters in experimental systems, both in vitro and in vivo. Although BFR effects on thyroid hormones are well confirmed, studies of effects on oestrogen/androgen systems are fewer but today growing in numbers. The effects of BFR on other hormone systems are still unknown. Hormonal effect levels in animals start from ca 1 mg/kg b.w., but there are exceptions: effects on spermatogenesis, suggesting hormonal causes, have been observed at a low dose (60 μg/kg b.w.) of a polybrominated diphenyl ether (PBDE) congener, BDE-99. It could be concluded that hormonal effects are of importance in risk assessment, and in some cases where effects are seen at low levels safety margins may be insufficient. One additional uncertainty is the lack of reliable human data that could be used to support animal BFR observations. In spite of the recent regulation of PBDE production, levels of both PBDE and of other BFR groups are still present in environmental samples. Thus, we have to deal with the possible effects of human BFR exposure for times to come. In order to reduce BFR exposure, the routes of exposure should be carefully examined and ways to reduce levels in major exposure routes considered.

BFR overview

Many types of chemical compounds are industrially used for flame-retardant purposes, and roughly one-fourth of these contain bromine. The global market demand for brominated flame retardants (BFR) was about 300 000 tonnes in 2000 (Alaee *et al.*, 2003). Among the large number of organobrominated flame-retardant compounds, a much smaller number of chemicals are used in larger volumes. Three of these groups are the polybrominated diphenyl ethers (PBDE), tetrabromobisphenol A (TBBPA) and its derivatives and hexabromocyclododecane (HBCD) (Fig. 1, Table 1). Even if the production of polybrominated biphenyls (PBB) has been stopped, these BFR are often mentioned because of their involvement in a large-scale poison scandal in Michigan in 1973–1974, where PBB was mistakenly mixed into animal feed (IPCS, 1994). In general, the chemical structure of and bromine presence in BFR make them persistent in the environment and in man, but differences exist depending on the type of BFR compound and degree of bromination. The higher brominated compounds tend to be less mobile in the environment, because of their low volatility, water solubility and strong adsorption on sediments, whereas the lower brominated compounds are predicted to be more water soluble and volatile, and also more bioaccumulative (Watanabe & Sakai, 2003).

The BFR have a number of applications, e.g. PBDE are mainly used in electronic equipment (e.g. TV sets, computers, circuit card boards), but also in building materials, in the transport sector and in electric appliances (Alaee *et al.*, 2003).

The occurrence of BFR in the environment has been followed in some matrices. In Sweden, a number of persistent organic pollutants (POPs) have been analysed in the yolk of guillemot since the beginning of the 1970s. In the case of PBDE, a rapid increase in levels was observed from the early 1970s up till about 1990, after which the levels turned down. Interestingly, the HBCD levels showed a similar increase during the same time period (1970–1990), but thereafter the increase continued till the end of the measurement period (Bignert *et al.*, 2007). In other countries, concentration–time trends for BFR may look different. In the USA and Canada, a continual rise

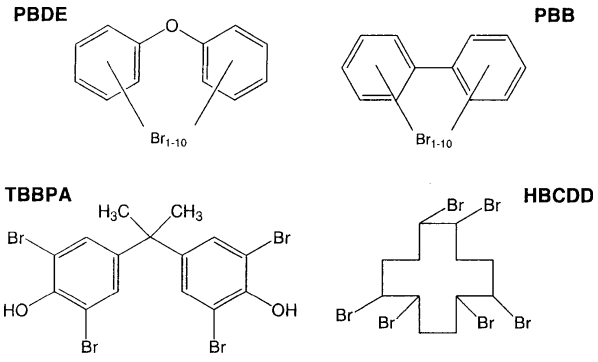

Figure 1 Chemical structures of brominated flame retardant groups.

Table 1. Estimated world market demand for TBBPA, PBDE, and HBCD in 1999 (from Watanable and Sakai, 2003)

Compound	Global volumes (metric tons)
TBBPA	121300
DecaBDE	54800
OctaBDE	3825
PentaBDE	8500
HBCD	15900

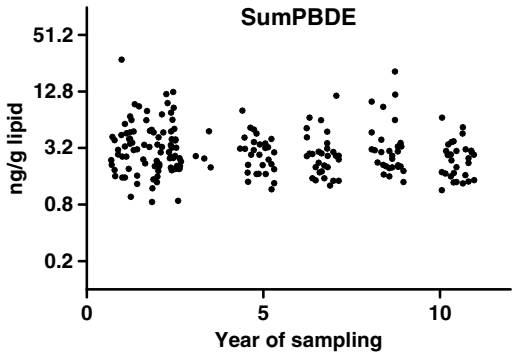

Figure 2 PBDE concentrations (sum of BDE 47,99,100,153) in mother's milk from primiparous mothers living in Uppsala County, Sweden (n = 211), starting Jan 1 1996 (year 0) and ending December 31 2006. Note that the plots are based on raw data that have not been adjusted for life-style factors and that the y-axis has a log scale (Glynn et al., 2007).

in PBDE levels has been reported in several matrices (e.g. Hale *et al.*, 2003).

In the case of PBDE, in 1998, a report showed a steep increase in breast milk levels in Swedish breast milk from 1972 to 1997 (Norén & Meironyté, 1998). Thereafter, measurements in many countries have confirmed the global presence of PBDE in human samples, analysing also blood serum and tissue biopsies (e.g. Bi *et al.*, 2006; Eslami *et al.*, 2006; Schuhmacher *et al.*, 2007; Toms *et al.*, 2007). The levels in human samples are generally considerably higher in the USA and Canada, as compared with both Europe and Asia (Schecter *et al.*, 2003; Hites, 2004; Ryan, 2004). Moreover, there is a continuing increase in the PBDE levels in the US breast milk (Schecter *et al.*, 2005), whereas the earlier increasing trend in Sweden has been replaced by a more steady-state type of situation (Lind *et al.*, 2003; Glynn *et al.*, 2007) (Fig. 2). For most people, food is suggested to be the main source of exposure (Darnerud *et al.*, 2001; Knutsen *et al.*, 2008), but other exposure routes such as inhalation/ingestion of dust could also contribute (e.g. Wu *et al.*, 2007).

Apart from a general background exposure, the BFR levels in humans may also be influenced by the occupational environment. Certain occupational groups handling BFR-containing computers or other electronic equipments may have higher tissue levels than people working in other occupations. Thus, in Sweden, dismantling workers were shown to contain substantially elevated levels of specific PBDE congeners that could be related to the type of congener pattern found in the material being handled at the place of work (e.g. Sjödin *et al.*, 1999; Jakobsson *et al.*, 2002). For example, very high levels of deca-BDE has been found in occupational electronic dismantling environments in certain regions in China (Qu *et al.*, 2007).

Regarding the toxic effects of BFR, results from earlier animal studies show (developmental) neurotoxicity, thyroid hormone effects and certain morphological effects in the liver and kidney. In the case of PBDE, only the full-brominated compound (deca-BDE) has been subjected to a long-time study in experimental animals, with no dramatic findings as regards carcinogenic potency (for review, see e.g. Darnerud *et al.*, 2001). Recent new findings suggesting oestrogen/androgen hormone-related effects have been reported (e.g. effects on sex hormone levels and of anogenital distance, puberty onset and impaired spermatogenesis; see Kuriyama *et al.*, 2005; Lilienthal *et al.*, 2006). In humans, no large-scale epidemiology study has so far been reported, and occupational studies are few and no clear effects found. Based on the effects so far found in experimental studies, an exposure margin for human exposure was estimated to range between 10 and 100 000 times, depending on BFR compound, type of experimental study, etc. (e.g. Darnerud, 2003).

BFR as endocrine disrupters

When it comes to endocrine effects of BFR, they may be caused by various brominated compounds, may affect various species and may be studied in different in vitro or in vivo models. These combinations thus result in a large number of possible variations that will take much effort and time to cover. However, some studies have

indeed shown endocrine-disrupting effects of BFR, and most of these studies concern effects on thyroid hormones (TH). In addition, there is an increased number of reports on effects on sex-related hormones, whereas effects on other hormonal systems are less studied (cf. review by Legler & Brouwer, 2003).

Effects on TH

The effects of BFR on TH have chiefly been studied in mammals but have also been observed in, for example, frogs and birds (Fernie *et al.*, 2005; Kudo *et al.*, 2006). In the frog, a TBBPA derivative affected triiodothyronine (T3) binding to TH-transporting protein and to hormone receptor, and in the American kestrel penta-BDE lowered the plasma thyroxine (T4) levels. In mammals, the most significant effect is often a decrease in thyroxin (T4), whereas T3 and TSH are less or not at all affected. Thus, in rodent, significant changes in plasma T4 is seen in exposed animals or in offspring after exposure to PBDE congeners or mixtures (10–18 mg/kg b.w.; Zhou *et al.*, 2001, 2002; Hallgren *et al.*, 2001), and occasionally also at comparably low levels (0.14–0.7 mg/kg b.w.; Fowles *et al.*, 1994; Andrade *et al.*, 2004; Kuriyama *et al.*, 2007). Moreover, T4 effects were also seen in rat offspring after exposure to HBCD (55 mg/kg b.w.; van der Ven *et al.*, 2006) and to TBBPA (from 16 mg/kg b.w.). A lower dose of TBBPA (5 mg/kg b.w.) gave no effects on TH in dams or offspring (Meerts *et al.*, 1999).

There are several possible mechanisms behind the BFR effects on TH. Effects could be caused by a direct attack on thyroid gland tissue, by alterations in the transport and metabolism/deactivation of TH, or by ligand binding to TH receptors or to other receptors with indirectly affects the TH homeostasis. Of these mechanism, effects of BFR directly on the thyroid gland are seldom seen, but a recent report on increased thyroid weight after HBCD exposure (benchmark dose, lower 95% confidence bound (BMDL) 1.6 mg/kg b.w./day) in rats could be noted (van der Ven *et al.*, 2006).

TH transport

Several studies show that BFR affect TH homeostasis by disturbing the peripheral TH transport. Hydroxylated metabolites of polychlorinated biphenyls (PCBs) and dioxins have earlier been shown to bind to the plasma TH transport protein transthyretin (TTR). The TTR binding will cause a displacement of the natural ligand, T4, and will result in a shorter half-time in the blood for T4 compared with the liganded form (Lans *et al.*, 1993; Brouwer *et al.*, 1998). Although there are differences in the importance of different classes of transport proteins between species, e.g. rat and man, TTR is of general importance for maternal and foetal transport of TH in vertebrates, and for transport of TH across the blood–brain barrier (Schreiber *et al.*, 1995). Furthermore, TTR is of importance in vitamin A transport, and compounds affecting TTR-mediated TH transport may also have an impact on the peripheral transport of vitamin A. In in vitro systems, TBBPA, bromophenols and PBDE metabolites have affinities to TTR that are of similar, or even higher, magnitude as T4 (Meerts *et al.*, 2000). On the other hand, in vivo experiments on TBBPA have suggested little or no effects on T4 levels in blood serum, a probable consequence of the rapid metabolism of TBBPA in vivo [in this case, the original compound and not the metabolites is the active, TTR-binding, structure (Meerts *et al.*, 1999)]. However, recent results in an in vivo screening model could show a TH-disrupting effect of TBBPA (Fini *et al.*, 2007).

TH metabolism

An alternative mechanism resulting in altered TH blood levels is the metabolism and deactivation of TH. T4 could be deactivated by phase II metabolism to its respective glucuronide conjugate. By binding to UDP-glucuronide transferase (UDP-GT), the thyroid hormone molecule cannot bind to the active site, and because of hydrophilic properties of the glucuronide structure, the conjugated hormone is instead eliminated by biliary secretion. Some exogenous compounds, among them dioxins and PCB, induce glucuronide conjugation enzymes, thus increasing the rate of this reaction (Brouwer *et al.*, 1998). Whether BFR induce glucuronide conjugation is an open question, and the rather limited effects found in rodent studies (e.g. Hallgren & Darnerud, 2002; Zhou *et al.*, 2002) may be an effect of contaminants present in the preparations. Another phase II enzyme that is suggested to be affected is iodothyronine sulfotransferase(s). Several POPs, including PBDE metabolites, inhibited sulfation of 3,3′-diiodothyronine in vitro, with could suggest interference with TH homeostasis also in the intact animal (Schuur *et al.*, 1998).

Receptor binding

Apart from the effects on transport and metabolism, some BFR compounds may actually affect TH-related receptor binding and activation. TBBPA has been reported to inhibit T3 binding to the TH receptor as well as to stimulate the proliferation of GH3 cells, a TH-dependent cell line (Kitamura *et al.*, 2002). Effects of TBBPA and its derivates on tadpole metamorphosis also suggest TH disruption by the inhibition of T3 binding to the TH receptor (Kitamura *et al.*, 2005a). In the case of PBDE, they might indirectly affect TH homeostasis by inducing the CYP enzymes 3a11

and 2b10 (Pacyniak *et al.*, 2007). These two enzymes are target genes of the pregnane x receptor (PXR), thereby suggesting that PBDE are activators of PXR. By inducing these CYP enzymes, more PBDE metabolites are formed, and it is known that PBDE metabolites affect TH transport (see 'TH transport' above!).

Consequences of TH disturbances?

What are the consequences of the BFR-induced disturbances of the TH homeostasis? Will not intricate feedback systems 'level out' the changes in TH that initially could be measured in different models, resulting in a lack of effects on physiological endpoints? Although this might be true for the adult individual, early developmental stages, within certain time windows, are suggested to be more susceptible and might be the ones first to be affected. Indeed, in vivo developmental toxicity models show that mice pups exposed to BFR (PBDE, HBCD) around day 10 after birth perform less well than the control animals in tests on spontaneous activity, spatial memory, etc. (Eriksson *et al.*, 2002; Viberg *et al.*, 2003), and that this decrease in performance often was persistent and seen long time after the exposure. The lowest doses giving effects in these experiments were in the range of 0.1–1 mg/kg b.w. (Sand *et al.*, 2004). On a body burden basis, the difference between levels giving effects in animal models and levels measured in high-exposed human populations may not be very large. The mechanism behind these developmental neurotoxic effects is not fully understood but could very well be TH dependent. This assumption is based on the above-discussed results of BFR on TH homeostasis and plasma TH levels, and the well-known fact that abnormal TH levels will affect mental development. The developing brain is dependent on TH for normal development, and abnormal TH levels will result in an impaired brain growth and differentiation, leading to mental retardation both in animals and in man (e.g. Haddow *et al.*, 1999; de Escobar *et al.*, 2004).

Another endpoint that could be affected by exposure to BFR is the hearing. By the same mechanism that impairs the general development of the brain, the development of hearing could also be affected (Crofton & Zoeller, 2005). Indeed, studies show that POPs such as PCB affect hearing in animals, via a TH-mediated mechanism, and in a recent study also the BFR compound TBBPA gave rise to a similar result, i.e. effects on brainstem auditory evoked potentials (BAEP) (Lilienthal *et al.*, 2008).

Effects on sex hormones

The effects of BFR on sex hormone receptor affinity has been observed in several in vitro systems (e.g. Meerts *et al.*, 2001; Kitamura *et al.*, 2005b; Hamers *et al.*, 2006; Cantón *et al.*, 2007; Harju *et al.*, 2007). In several of these systems, antagonistic properties on sex hormone receptors were observed: in the androgen receptor (AR)–Calux model (Hamers *et al.*, 2006), the most pronounced AR antagonism was found for the PBDE congeners BDE-19 and BDE-10, and in a yeast, bioassay AR antagonism was also found for several hydroxylated BDE metabolites (Cantón *et al.*, 2007). The Calux study also showed (although with low potency) oestrogenic responses for some low-brominated diphenyl ethers up to the hexabrominated BDE congeners, anti-oestrogenic activity for some phenolic brominated compounds, the most potent compound being 6-OH-BDE-47. In the same study effects on oestradiol sulfation by oestradiol sulfotransferase (resulting in increased circulating oestradiol levels) were shown to occur following exposure to TBBPA, and another study (Kitamura *et al.*, 2005b) could show anti-oestrogenic potency of TBBPA in an MCF-7 cell line. Finally, the in vitro results by Meerts *et al.* (2001) suggest that several pure PBDE congeners, and especially PBDE metabolites and brominated bisphenol A analogues, are agonists of both ER-alpha and ER-beta receptors.

In vivo studies reporting on sex hormone effects from BFR exposure are still rather few but the area is expanding. In a study on rats, Lilienthal *et al.* (2006) studied the effects on sex hormone levels in rat offspring after exposure to the PBDE congener BDE-99 (1 or 10 mg/kg b.w./day) during pregnancy days 10–18. The two BDE-99 doses gave dose-dependent, significant decreases in oestradiol levels in male offspring at three weeks after birth, and at an even longer time after birth (160 days), the testosterone levels in the BDE-99-exposed male offspring were decreased (although significant only for the 10 mg/kg dose). In addition to the observed changes in plasma oestradiol and testosterone, BDE-99 also increased sex hormone related sweet preference and although not significantly reduced anogenital distance in male offspring, both effects indicating a feminization effect of the BDE congener. Moreover, a delayed puberty onset in female offspring was observed at doses of 1 or 10 mg/kg b.w., to the dam (Lilienthal *et al.*, 2006). Using a similar methodological approach, Talsness *et al.* (2006) studied the effects of rat offspring after BDE-47 exposure during pregnancy (140 or 700 μg/kg b.w., single oral dose on gestation day 6). In addition to a decrease in ovarian follicular counts in the F1 offspring, the concentration of circulating oestradiol was significantly decreased in female offpring belonging to the high-dose group. The same experimental protocol (BDE-47 as a 140 or 700 μg/kg b.w., single dose to rats during pregnancy) resulted in a decrease in serum concentrations of follicle stimulating hormone (FSH) at the higher dose, in male offspring rats at post-natal day 22 (Andrade *et al.*, 2004).

In an in vivo study on TBBPA by van der Ven *et al.* (2008), the compound was given in the diet to rats in eight dose groups (0–3000 mg/kg b.w./day; from before mating, and in dams during gestation and lactation) in a one generation reproduction study. Analysis on testosterone and 17β-oestradiol in the plasma of F1 males showed no dose-dependent effects, nor did CYP19 activity in ovaries of F1 females (CYP19 = aromatase, microsomal enzyme active in oestradiol metabolism). However, the reproductive organs of male F1 pups at weaning showed an increase in weight already at a benchmark dose (BMDL) of 0.5 mg/kg b.w./day, and both the testosterone levels and CYP19 activity were correlated to the increased testis weight, suggesting a similar mechanism for the TBBPA action on these endpoints.

In other animals than mammals, BFR-related effects on sex hormones have so far been difficult to show. In birds, the development of Mullerian ducts is dependent on oestrogen levels, and in Japanese quails exposed to TBBPA, no malformations of these ducts were observed (Berg *et al.*, 2001). In fish, exposure to PBDE or TBBPA gave no effects on oestrogen-dependent vitellogenin levels (Christiansen *et al.*, 2000; Boon *et al.*, 2002).

In mammals, in addition to direct hormone measurements, other hormone-related effects of BFR have been observed. In rats, impaired spermatogenesis, measured as reduced sperm counts, has been observed in male offspring upon a single, low dose of 60 μg BDE-99/kg b.w. to the dam on day 6 of gestation (Kuriyama *et al.*, 2005). In addition, hyperactivity was observed in offspring already at the lower dose. These are so far the most sensitive effects found as response to PBDE exposure. The internal tissue BDE-99 levels corresponding to the effects seen in the mentioned rat study was reported separately (Kuriyama *et al.*, 2007). Interestingly, it was shown that the PBDE levels in the rat adipose tissue, corresponding to the 60 μg/kg b.w. dose, were only about three times higher than the maximum breast milk levels observed in a US study, and 22 times higher than the mean level from the same study. Another study suggesting a sex hormone-related effect is the morphological changes seen in the ovaries of rat offspring after similar gestational exposure (Talsness *et al.*, 2005). In this study, the dissolution of mitochondria and increase in vesicles may indicate disruption of steroid synthesis. In a study using a commercial PBDE mixture, DE-71, a delay in pubertal progression and changes in androgen-dependent tissues (decreased seminal vesicle and ventral prostate weights) was found in male rats after a 30-day exposure to 30 and 60 mg/kg b.w./day (Stoker *et al.*, 2004). In a subsequent paper, the findings of effects on androgen-dependent tissues were further explored in order to elucidate possible mechanisms (Stoker *et al.*, 2005). By the use of both in vivo and in vitro methods, the observed effects by the PBDE mixture DE-71 were suggested to be caused by an inhibition of AR binding by several congeners present in the mixture. In a separate study, changes in the sexual behaviour (strong impairment of lordosis quotient and incentive behaviour) have been observed in female offspring after PBDE exposure to dams GD 10–18 (10 mg/kg b.w./day) (Lichtensteiger *et al.*, 2004).

In the above studies on the effects on sex hormones and related endpoints, some suggestions are made on possible mechanism behind the observed effects. In in vitro studies, both agonistic and antagonistic effects on binding to sex hormone receptors have been defined. These mechanism may also act in vivo, and indeed in the study by Stoker *et al.* (2005) the effects of the PBDE mixture DE-71 on male rats (puberty delay and decreased growth of androgen-dependent tissues) were suggested to be caused by androgen receptor (AR) binding by several of the congeners which make up the mixture. In a study on BDE-99 on rats, offspring uterine hormone (progesterone, oestrogen alpha, beta, IGF-I) receptor levels were affected, suggesting that developmental PBDE exposure could interfere with the regulation of oestrogen target genes in uterus (Ceccatelli *et al.*, 2006). Alternate mechanisms, acting secondary to a decrease in thyroid function that can influence steroidogenesis or by direct action of the compounds on target tissues, may although still be valid in certain cases. Thus, the observed changes in follicle numbers observed by Lilienthal *et al.* (2006) were suggested to be related to thyroid effects, but other mechanisms were not excluded. In the study where structural changes in the ovaries of rats were observed, the effects of BDE-99 on the mitochondria (altered mitochondrial regulation) were suggested to explain the effects (Talsness *et al.*, 2005). Finally, the strategy of using statistical methods for clustering of parameters based on result for different endpoints, as shown by van der Ven *et al.* (2008) for TBBPA, may be a useful tool in ongoing studies on mechanisms for the effects of BFR on sex hormones, but also on other hormonal systems.

Possible effects on other hormonal systems?

No in vivo effects of BFR on other hormonal systems than those described above have been found in the literature. However, in an in vitro study by Ding *et al.* (2007), effects of brominated flame retardants and brominated dioxins were observed on steroidogenesis in a human adrenocortical carcinoma cell line. The brominated compounds affected gene expression of several steroid-regulating proteins, suggesting a modulation of steroidogenesis and a possibility for endocrine disruption.

Effects of BFR in humans

As already mentioned, no large epidemiological study on BFR has to this date been presented. The studies that are available are small in size and contain deficits, e.g. as regards their exposure data. In an early occupational exposure study (Bahn *et al.*, 1980), using a coarse exposure estimation, there was some evidence of hypothyroidism after mixed exposure to PBDE and PBB. In a Japanese study, 12 pregnant women were followed, and at birth both maternal and umbilical cord blood was taken both for TH analysis and for measurement of PBDE levels (Mazdai *et al.*, 2003). No correlation between PBDE and TH levels was found. In a study of consumers of fish containing persistent organic pollutants, no firm correlation between levels of analysed contaminants, including BDE-47 and TH, and other hormone levels, was found (Hagmar *et al.*, 2001).

In a recent report from a prospective Danish–Finnish study (performed in 1997–2001), boys were examined for cryptorchidism, mother's milk and placentas were analysed for PBDE congeners, and infant serum for several sex hormones (Main *et al.*, 2007). The PBDE levels (seven major congeners) in breast milk, but not in placental tissues, showed an association with congenital cryptorchidism. There was also a positive correlation between levels of PBDE in milk and serum luteinizing hormone (LH).

Overall conclusions

As described in this review, BFR are endocrine disrupters in experimental systems, both in vitro and in vivo. In the case of TH, the effects of certain BFR groups, especially PBDE, are well confirmed. As regards oestrogen/androgen effects, the studies are fewer but effects are nevertheless described, some occurring at an unusually low dose. Few human studies have been performed, one showing a correlation between PBDE in breast milk and congenital cryptorchidism. The effects of BFR on other hormone systems are unknown. In spite of the recent regulation of PBDE production, levels of both PBDE and of other BFR groups are still present in environmental samples. Thus, we have to deal with the possible effects of human BFR exposure for times to come. In order to reduce BFR exposure, the routes of exposure should be carefully examined and ways to reduce levels in major exposure routes considered.

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Panel discussion

J. McLachlan

Is the primary mechanism of thyroid disruption by these flame retardants through an effect on the thyroid binding proteins rather than direct interaction with the thyroid hormone receptor?

P.O. Darnerud

Most studies suggest that there is binding to the transport proteins which is probably the main effect although other mechanisms cannot be excluded. These results are based on animal experiments, but the situation may differ in humans because of different transport proteins.

G. Lyons (UK)

I am worried about the potential developmental neurotoxic effects of these brominated flame retardants especially Deca PBDE, and possibly TBBPA. All the developmental neurotoxins which are well characterized, such as lead, mercury and polychlorinated bisphenols, have been shown in epidemiological studies to act in humans at levels which are around three orders of magnitude lower than would have been expected from the initial rodent studies. Rodents seem, therefore, not to be good protective models for this endpoint unless we accept greater assessment factors for extrapolation between species. It would not be prudent to wait for epidemiological studies to show an effect if exposure prevention could be instigated at an earlier stage.

P.O. Darnerud

We do see effects in animals and there are differences between man and animals. At present I cannot be sure of the impact on the human population.