Maisons-Alfort, 24 July 2006

THE DIRECTOR GENERAL

OPINION

of the French Food Safety Agency regarding the assessment of risks related to the presence of brominated flame retardants in food

On 31 March, 2005 the Directorate General for Food requested that the French Food Safety Agency (AFSSA) assess the risks related to the presence of brominated flame retardants in food in order to identify the relevant analyte-matrix pairs which should be analysed to better determine the exposure of the French population to these contaminants.

After consulting the Scientific Panel of Experts on 'Physical and chemical contaminants and residues' (RCCP), which met on 7 June and 5 July 2006, the French Food Safety Agency reached the following conclusions.

1 BACKGROUND

Brominated Flame Retardants (BFR) are chemical products added to plastics used in electrical equipment (computers, televisions) and electronic circuitry for their flame retardant properties. They are also found in foams and padding materials (residential and industrial), automobile and aircraft interiors as well as in some textiles. Their mode of action is based on trapping the active radicals produced during the gas phase of the combustion.

Included among existing BRFs are:
- tetra-bromo-bisphenol A (TBBPA)
- hexa-bromo-cyclododecane (HBCD)
- polybrominated diphenyl ethers (PBDE) of which only deca-BDE is authorised in Europe
- polybrominated biphenyls (PBB), banned in Europe and no longer produced since 2000.

The chemical structure of these compounds or families of compounds is shown in Annex 1.

World production of brominated fire retardants reached approximately 200,000 tons in 2003 (BSEF, 2006), including nearly 60% in the form of TBBPA, over 30% in the form of PBDE and 5 to 10% as HBCD.

PBDEs are used as additives during production of these manufactured goods and therefore are likely to move around in the polymer matrix and be rejected in the process, unlike so-called reactive brominated flame retardants (TBBPA), which are related structurally to the matrix in which they are impregnated.

PBDEs are obtained by bromination of diphenyl ether, with the synthesis conditions determining the degree of halogenation of the molecules. Until 2004, the authorised commercial blends of PBDE that were in wide use on the market were deca-BDE (containing a small percentage of octa- and nona-BDE), octa-BDE (actually a mixture of octa- and hepta-BDE) and penta-BDE.

Penta-BDE, octa-BDE and deca-BDE are included in the European programme for risk assessment on Existing Substances; penta- and octa-BDE have been classified by the European Union:
- penta-BDE is classified as harmful (Xn, R48/21/22 and R64: possible risk of harm to breastfed babies) and also very toxic to aquatic organisms (N, R50-53);
- octa-BDE is classified as toxic (T, R61: may cause harm to the unborn child during pregnancy and R62: possible risk of impaired fertility).

No classification has been designated for deca-BDE.

Moreover, the marketing of products or articles that contain concentrations of more than 0.1% by mass of octa-BDE and penta-BDE has been prohibited in the European Union since 15 August 2004 (Directive 2003/11/EC). Consequently, only deca-BDE is still authorised. However, even though the use of penta-BDE and octa-BDE no longer concerns the European Union directly, it has been estimated that these mixtures account for around 25% of global PBDE production (Damerud, 2001).

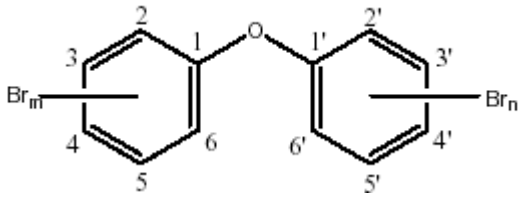
Due to their widespread use and their characteristic persistency, BFRs can end up in the environment and contaminate the food chain.

To assess risks for consumers related to the presence of brominated flame retardants in foodstuffs, a review of current knowledge has been made regarding both the effects of these compounds on health and contamination of foodstuffs, and population exposure. An investigation concerning PBDEs alone has been undertaken, focussing on the following points:

- presentation of the main toxicological data on PBDEs while highlighting the inadequacy of the data for defining a toxicological reference value,
- known sources of exposure and data on food contamination available in the literature in France and elsewhere.
- levels of impregnation reported in the literature,
- identification of analyte-matrix pairs to adapt the sampling of a surveillance/control plan,
- identification of an analytical method

2 THE STRUCTURE OF PBDES

PBDEs are organohalogenated compounds with a common diphenyl ether backbone where each of the aromatic cycles is substituted by 0 to 5 bromine atoms, that is to say between 1 and 10 bromines for the whole molecule. Including isomers, this family of compounds consists theoretically of 209 different compounds (IUPAC PBDE nomenclature: Annex 2), exactly as for the PCBs (polychlorobiphenyls). Just as for PCBs, the number of isomers actually present in the specialty products synthesised by the industry is less than 209.



PBDE

PBDEs are compounds that are relatively stable physically and chemically. Depending on the degree of bromination, their boiling point is between 310 and 425°C. These molecules are not very water soluble, especially the more brominated congeners. Their octanol-water partition ratio or K_{ow} ranges between 4.3 and 9.9, which promotes their bioaccumulation in fatty tissue. There is very little data on the environmental fate of BFRs from European studies currently in progress (EU FIRE project). Compounds of high molecular weight, deca-BDEs, but also nona- and octa-BDEs, are supposed to have a low potential for bioaccumulation. It is known, however, that when subject to UV radiation, deca-BDEs rapidly lose one or more bromine atoms and consequently may be degraded to produce the entire series of polybromodiphenyl ethers as well as corresponding furans (Watanabe *et al.*, 1986; data recently confirmed by Soderstrom *et al.*, 2004; Debrauwer *et al.*, 2005).

3 ROUTES OF EXPOSURE FOR HUMANS

There are three potential routes of exposure to PBDE via:
- ingestion of contaminated food,
- absorption through the skin (contact with materials or fabric containing PBDEs),
- inhalation.

For the two latter routes, the cases of people exposed in the course of occupational activity (plants manufacturing or dismantling electronics products, etc.) should be considered separately from the general population and will not be dealt with here.

For the general population in Europe, food would be the primary vector for PBDE exposure. However, exposure through the air should be considered in light of recent publications reporting the presence of PBDEs, especially high molecular weight PBDEs, in household dust. At the moment the data are mainly available for North America (Stapleton, 2005). The presence of PBDE in dust may be due as much to airborne contamination as to the use of these compounds in various household materials, including upholstery fabrics, rugs and carpets. Currently, it is very difficult to estimate the impact of these findings and to assess the relative importance of this contamination source compared with that of food intake. Especially since the residual levels of PBDE are considerably higher in North America than in Europe.

4 TOXICOLOGICAL DATA

Several agencies have conducted analyses of PBDEs, primarily based on data published in the literature (IPCS - WHO, 1994, ATSDR, 2004, JECFA, 2005, UK-COT, 2004). Although these chemicals have been studied as part of registration applications, particularly under 67/548/EEC concerning new substances and Regulation (EEC) No. 793/93 on the assessment and control of risks involved with existing substances, these studies are not available in open literature.

Most of the available toxicological data are old and come primarily from the manufacturers of products (Darnerud, 2003, Gill *et al*., 2004). Most involve non standardised studies that were frequently conducted without regard for Good Laboratory Practice (Hardy, 2002). Furthermore, these studies generally concern commercial mixtures of PBDEs, rather than isolated PBDEs. Unless otherwise stated, the paragraphs below show the established results for commercial mixtures, for which the respective proportions of various PBDEs are indicated when they are mentioned in the literature.

The toxicological data in this document include the essential points taken from analysis of the JECFA and ATSDR records and from a review of PBDEs by Darnerud, published in 2001. The draft report written for the reassessment of existing substances ((EC) Regulation No. 793/93), available on the European Commission site, does not add anything new to the assessments of JECFA and ATSDR.

4.1 Kinetic Data

Absorption

The first study suggesting quantitation of the absorption of deca-BDE was done in rat, following oral administration of 1 to 5000 mg/kg of [^{14}C]-labelled deca-BDE (El-Dareer *et al*. 1987). The authors found a weak absorption of deca-BDE, with the residues detected in faeces and intestines accounting for 99% of the dose 72 hours after administration, whereas the liver contained 0.45% and all tissues less than 1%. The findings of this study, as well as previous results (Norris *et al*., 1975; NTP, 1986), have long been used as an argument in favour of a very low bioavailability of deca-BDE (i.e. less than 1%), although there is no evidence for excluding a greater absorption of deca-BDE, in particular, through hepatic biotransformation and biliary excretion of the metabolites formed.

Other more recent data on the oral route clearly demonstrate that the bioavailability of deca-BDE is higher in rat. In 2003, experiments using 2 mg/kg doses found a minimum bioavailability of 10%, for Mörck *et al*., and 26%, for Sandholm *et al*.

The data on biliary excretion of deca-BDE, which varies but is about 10% of the administered dose (El-Dareer *et al*., 1987; Mörck *et al*., 2003), also indirectly confirm bioavailability significantly greater than 1%.

As part of the AFSSET RD-2004-011 programme, a study of metabolism was conducted in gravid Wistar rat receiving a daily administration of 1.95 mg/kg of [14C]-deca-BDE orally, from D16 to D19 of gestation; metabolic analyses indicated absorption above 18% (radioactivity found in the tissues and the carcass) which confirms that a significant proportion of the deca-BDE is absorbed in rat after administration by oral route (Riu *et al*., 2006).

Concerning the other PBDEs, the available results suggest that oral absorption is greater than that of deca-BDE, in inverse proportion to the number of bromine atoms carried by the diphenyl ether structure. However, there is scant data on pure PBDE and the oldest studies were only conducted with commercial mixtures:

- after oral administration of tetra-BDE (BDE-47) to rats and mice, Orn and Klasson-Wehler (1998) showed that for both species, tissue residues accounted for 80% of the dose administered, indirectly indicating very good absorption;
- for penta-BDE, the minimum bioavailability of a commercial mixture of congeners (DE-71) administered by oral route to Sprague-Dawley rats has been estimated at more than 36% (liver and carcass) (Hakk *et al*., 2001; Hakk and Letcher 2003). These data have recently been upheld by a more specific study of BDE-100 (Hakk *et al*., 2006), showing that 72 hours after administration of the radiolabelled chemical, over 70% of the administered dose was found in the tissue;
- for octa-BDE (commercial mixture DE-79), a study done in rat by the same team showed that at least 23% of the administered dose was found in the tissues (liver and carcass) of rats exposed by oral route for a period of 21 days (Huwe *et al*., 2002).

Distribution

Concerning deca-BDE, the reference study for the oral route in rat (Sprague-Dawley, males) is that of Mörck *et al*. (2003). After administration of a single dose of 2 mg/kg of [14C]-deca-BDE, these authors showed that the highest residual levels were found in the liver (*ca.* 0.5 ppm), but especially in the adrenal glands (*ca*. 1.2 ppm) three days after administration. Values near 0.15 and 0.10 ppm were determined respectively for kidney and adipose tissue, while the residual levels in all other tissues were lower than 0.07 ppm.

Recent results reported by Riu *et al*. (2006) after administration of repeated doses of deca-BDE (1.95 mg/kg) to Wistar rats during gestation confirm that the target tissue is the liver (receiving more than 6% of the administered dose, which is greater than 11 ppm of equivalent deca-BDE). The residual concentrations measured in the adrenal glands were even higher (33 ppm) and the ovaries were also a target tissue (16 ppm). The residual levels detected were lower (0.1 to 3.9 ppm). In addition, between 0.4 and 0.5% of the radioactivity administered was found in the foetuses (of the whole litter) and in the placenta (aggregate of placentas).

Contrary to what has been observed for the deca-BDEs, adipose tissue appears to be an important storage site for less brominated congeners (Örn and Klasson-Wheler, 1998; Hakk *et al*., 2002; Staskal *et al*., 2006). However, the studies currently available do not enable, for the most part, determining whether the compound is retained in its parent form or in metabolite form.

In mouse, after injection of [14C]-BDE-47, -BDE-85 and -BDE-99, radioactivity is concentrated in adipose tissue, the liver, adrenals, ovaries, lung and brain. Sixteen days after injection, radioactivity is primarily found in the adipose tissue, liver and lung. The distribution does not appear to differ according to congeners. In the case of gravid mice, passage to the fœtus seems low but has not been quantified. In contrast, in lactating mice, injection of a mixture of penta-BDE ([14C]-BDE-85 and -BDE-99) shows a higher passage into milk and a transfer of 20% of the dose to all of the litter (Darnerud *et al*., 2006). In rat, the induction of liver enzymes and lower levels of T4 in fœtuses indicate transplacental passage when dams are exposed to a commercial mixture of tetra- and penta-BDE (DE 71) (Zhou *et al*., 2002).

Metabolism

After oral administration of [14C]-deca-BDE in rats, Sandholm *et al*. (2003) showed 13 plasma metabolites, including hydroxylated and/or methoxylated metabolites of nona-, octa-BDE and hexa-BDE. The exact structures of the formed metabolites (position of oxidation and/or debromination) could not be established formally, given the analytical difficulties. Similarly, they could not be quantified. The study by El-Dareer *et al*. (1987) had suggested that residues of deca-BDE excreted in faeces contained 1 to 28% residues of the metabolites (unidentified) of deca-BDE with a possible involvement of bacterial biotransformations, particularly for the debromination processes. However, the identification of oxidative metabolism routes of appears to indicate metabolism by hepatic P450 cytochromes. Hydroxylated metabolites have also been found in rat blood plasma following intraperitoneal administration of a mixture of PBDE (Malmberg *et al*., 2005).

Among the residues of deca-BDE excreted in the faeces 3 days after exposure, 65% of the dose was found in the form of metabolites, including 8 phenolic compounds, but this has not enabled the structure of these metabolites to be characterised to date (Mörck *et al*., 2003).

The first radio-chromatographic profiles of tissue extracts taken from rats treated with [14C]-deca-BDE have only been made available recently (Riu *et al*., 2006). They show the presence of 5 to 15% of metabolites in different tissue compartments, as well as in fœtal compartments.

Deca-BDE is eliminated rapidly in rat. The half-life, following intravenous administration, has been estimated at 58 hours (Sandholm *et al*., 2003). In man, the half-life of deca-BDE is estimated to be between 7 and 14 days.

In the case of penta-BDE (BDE-99), faecal metabolites were partially identified as 2 mono-OH-penta–BDE and 2 mono-OH-tetra–BDE, indicating debromination *in vivo*. The presence of a non-extractable fraction of residues suggests the formation of covalently bound reactive intermediaries (Hakk *et al*., 2002; Hakk and Letcher, 2003).

Concerning the tetra-BDE (BDE-47), 6 hydroxylated metabolites were detected in the faeces and tissues of rodents, with the parent compound remaining predominant (Orn and Klasson-Wehler, 1998). After administering BDE-47 orally to the rats, chromatographic analysis of the faeces enabled the identification of 6 tetra-OH-BDE and 3 tri-OH-BDE (Marsh *et al*., 2006). In mouse, excretion is not limited to the faecal route since a third of the dose is found in urine 5 days after exposure, against less than 1% in rat (Orn and Klasson-Wehler, 1998; Staskal *et al*., 2006).

The half-lives of tetra-BDE, after oral administration in mouse, have been estimated at 1.5 d (t1/2a) and 23 d (t1/2b), the latter value suggesting potential bioaccumulation (Staskal *et al*., 2006).

Kinetics – key points

To conclude, there are still far too few studies which have addressed the (toxico)kinetics of PBDE. They concern only a limited number of congeners, often studied in combination, emphasising a bioavailability that varies according to the degree of bromination, a tendency for less brominated compounds to be retained in adipose tissue, and high residual levels of deca-BDE in the adrenals and ovaries. These studies also indicate the presence of hydroxylated and methoxylated metabolites, whose exact structure and presence in different tissues could not generally be determined.

The following points should be emphasised:

- the possibility that PBDE in general (Hakk *et al*., 2002; Marsh *et al*., 2006), and deca-BDE in particular (Sandholm *et al*., 2003; Mörck *et al*., 2003, Riu *et al*., 2006) can undergo debromination *in vivo* cannot be ruled out in view of studies done in rats. However, the analysis of PBDE in foods and in humans frequently involves compounds that have 4 to 7 bromines;
- the corresponding metabolites have seldom been sought;
- profiles of tissue distribution are not available with the exception of a study done in gravid rats during gestation;

- there are not enough data on placental transfer as well as passage into milk (plasma/milk ratio not indicated); in the Wistar rat, approximately 1% of an oral dose of 1.95 mg/kg was found in fœtuses (0.4%) and placentas (0.5%) (Riu *et al.*, 2006);
- there is also not enough data on the respective tissue and elimination half-lives of each PBDE. However, it may be noted that for congeners with 4 to 6 bromines, the half-life seems to increase in direct proportion to the number of bromines, whereas for congeners with 6 to 8 bromines, the half-life decreased when the number of bromines rose (ATSDR, 2004);
- there are no data for determining the efficiency of pulmonary absorption for deca-BDEs, while an airborne route of exposure might be possible (cf. sources of exposure);
- methoxylated metabolites of PBDEs, which have been found in species of wild fish (Sinkkonen *et al.*, 2004) and marine mammals (Marsh *et al.*, 2005), might not only be products of biotransformation of PBDE, but also compounds of natural origin, generated in the marine biotope (Vetter *et al.*, 2001). Understanding their formation mechanism is thus essential to be able to estimate the extent of exposure linked unequivocally to anthropogenic sources;
- the presence of substantial residual levels in the adrenal glands (Mörck *et al.*, 2003; Riu *et al.*, 2006) and in the ovaries (Riu *et al.*, 2006), in rat, after oral administration of deca-BDE, raises additional questions given the hypothesis of there being biologically active parent compounds or metabolites present.

4.2 Acute toxicity

Oral DL50 in rats is high: above 5 g/kg for deca-BDE, above 10 g/kg for octa-BDE and between 0.5 and 5 g/kg for penta-BDE with effects such as slower growth, diarrhoea, piloerection, tremors, preocular and perinasal redness, and damage to the liver and gastric mucosa (Norris *et al.*, 1975; IPCS 1994; JECFA 2005).

Deca-BDE does not irritate the skin and octa-BDE is a mild irritant.
Concerning the respiratory route, no mortality has been observed in rats exposed for 8 hours/day for 14 days to dust with an octa-BDE concentration of 174 mg/m^3. The observed effects include increased respiration rate (reversible), mild nasal irritation without histological alterations of the lungs or liver impairment (ATSDR, 2004).

4.3 Subchronic and chronic toxicity

Various studies from 14 days to 103 weeks have been conducted by oral route in rodent (rats or mice) with deca-, octa- and penta-BDE. Toxic effects were mainly observed in the liver, kidney and thyroid.

However, since the available studies concern only a limited number of congeners and a large portion of those were for PBDE mixtures, it is not possible to attribute a particular effect to a particular congener. In addition to these, there are some studies that cannot be interpreted because the exposure amounts are expressed by kg of food but fail to specify the dose ingested. Table 1 shows the results of the main toxicology studies conducted on PBDEs.

Table 1: Main toxicology studies on PBDEs

PBDE Congener	Species, study duration	Critical effect	NOAEL	LOAEL	References

PBDE Congener	Species, study duration	Critical effect	NOAEL	LOAEL	References
Deca-BDE 77.4% + 21.8% of nona-BDE + 08 % deca-BDE	Sprague Dawley Rat 30 days	Liver damage, thyroid hyperplasia	8 mg/kg b.w./day	80 mg/kg b.w./day	Norris, 1973; Norris *et al.*, 1975
Deca-BDE (94-99%)	B6C3F1 Mouse 103 wks	Liver and thyroid hypertrophia	< 3200 mg/kg b.w./day		NTP, 1986
	Fisher Rat 344/N 103 wks	Liver damage, pre-stomach acanthosis, impairment of the pancreas, spleen and lymphoid tissue	< 1200 mg/kg b.w./day		NTP, 1986
Deca-BDE (97%)	Female SD Rat 20 days	No toxic effect observed in foetuses or dams	1000 mg/kg b.w./day		Hardy *et al.*, 2002
Octa-BDE	Rat SD 30 days	Histological lesions of the liver and kidney, thyroid hyperplasia	-	8 mg/kg b.w./day	Norris, 1973; Norris *et al.*, 1975
Penta-BDE and tetra-BDE commercial mixture DE-71 58.1 % penta 24.6 % tetra	B57BL Mouse 14 days	Increase in relative weight of the liver and thymus, decreased T4	36 mg/kg b.w./day	72 mg/kg b.w./day	Fowles *et al.*, 1994
	Wistar Rat males 31 days females 20 days	Induction of phase I and II liver enzymes, delayed sexual maturity, decreased T4	3 mg/kg b.w./day	30 mg/kg b.w./day	Stoker *et al.*, 2004
	Long Evans Rat Long Evans females 36 days	In dams and pups: Induction of phase I and II liver enzymes, decreased T4	1 mg/kg b.w./day	10 mg/kg b.w./day	Zhou *et al.*, 2002
Tetra-BDE	Female SD Rat and B57BL Mouse 14 days	Decreased T4, liver vitamin-A, induction of phase I enzymes		18 mg/kg b.w./day	Hallgren *et al.*, 2001

NOAEL: no observable adverse effect level; LOAEL: lowest observed adverse effect level

The studies do not indicate the actual doses without toxic effects. There is only one value of 8 mg/kg b.w. /day from a 30 day study in rats with a mixture of deca-, nona- and octa-BDE but with only 5 animals per gender per dose. Toxic effects were observed at doses from 80 mg/kg b.w. /day for a mixture of deca- and nona-BDE, from 80 mg/kg b.w. /day for octa-BDE and from 18 mg/kg b.w. /day for tetra-BDE (references in Table 1). Toxicity tends to decrease with an increase in the number of bromines.

In almost all studies, liver symptoms occur at lower doses than those in which thyroid damage has been observed, with toxicity being more pronounced in male animals. The liver effects result essentially in hypertrophy and vacuolisation of hepatocytes, some foci of necrosis, pigmentation of Küpffer cells and adenocarcinomas. Effects on the thyroid are characterised by hypertrophia/hyperplasia of follicular cells and adenocarcinomas. The link between toxic effects in the liver and thyroid is quite common in rodent and particularly in rat due to the inductive effect on liver enzymes of the xenobiotics.

An inducing effect of PBDEs was observed for phase I enzymes (cytochrome P-450) *in vitro* (Chen *et al.*, 2001) and *in vivo* in mice, from 18 mg/kg b.w/day of tetra-BDE (Hallgren *et al.*, 2001). An induction of UDPGT [Uridine Diphosphate Glucuronyltransferase] was also reported in the pups of parents exposed to 30 mg/kg b.w. /day of a commercial mixture (DE 71) of tetra- and penta-BDE (Zhou *et al.*, 2002; Stoker *et al.*, 2004).

Some other effects should be clarified, especially reductions in primary erythrocyte parameters as well as the occurrence of porphyria, which is particularly evident with penta-BDE.

Studies indicate some impaired immune functions. Nevertheless, it is advisable to be cautious when interpreting the results because the effects observed in animals in this area are rarely predictive of what may be observed in humans. In addition, repeated dose studies have apparently not indicated any symptoms involving target organs such as the spleen, thymus, or lymph nodes. In the NTP study (1986) a fibrosis of the spleen and hyperplasia of lymphoid tissues were observed at a high dose of deca-BDE in males (2240 mg/kg b.w. /day, 103 weeks). However, these effects were obtained with very high doses disproportionate to the dose to which humans might be exposed.

A higher sensitivity in fœtuses and newborns has been noted in many rodent studies, with neurological effects detected in adulthood (hyperactivity, altered spontaneous behaviour) (Branchi *et al*., 2003; Kuryama *et al*., 2005; Staskal *et al*., 2006; Viberg *et al*., 2006).

Concerning the respiratory route, the data are insufficient. However, this route should not be overlooked given the presence of PBDE in household dust (Stapleton, 2005). The toxic effects observed in studies conducted for 13 weeks in rats exposed to octa-BDE (1.1 to 202 mg/m^3), include mucous cell hyperplasia, chronic alveolar inflammation, liver impairment and lymph node damage, decreased plasma T4 levels and an increase in TSH levels (ATSDR, 2004).

4.4 Genotoxicity

The little information that is available essentially concerns deca-BDE, which does not appear to have genotoxic potential *in vitro*. In *in vitro* mutagenesis tests, deca-BDE does not induce gene mutations on 4 bacterial strains (TA98, TA100, TA1535 or TA1537) or mammal cells (mouse lymphoma). No induction of sister chromatid exchange or chromosome aberrations (CHO cells) has been observed (NTP 1986).

For penta-BDE (BDE-99), mutagenicity tests on *S. typhimurium* (TA98, TA100) and *E. coli* (WP2 uvrA) and clastogenicity tests on *A. cepa* were negative (Evandri *et al*., 2003).

The only reported positive results come from the study by Helleday *et al.* (1999), in which the *in vitro* exposure of Chinese hamster cells SPD8 and Sp5 V79 to tetra-, di- and mono-BDE (BDE-47, BDE-12 or BDE-1) increased the genetic recombination at the HGPRT locus. However, interpretation of this type of recombination requires clarification (Darnerud *et al*., 2001; JECFA, 2005).

In its monograph, the IPCS (1994) presents negative *in vitro* results for octa-BDE (commercial mixture) in studies carried out in 1987, not presented in detail and concerning the non-programmed synthesis of DNA on human fibroblasts (WI-38), gene mutation on *S. typhimurium* and *Saccharomyces cerevisiae* and sister chromatid exchange in Chinese hamster ovary cells. Negative *in vitro* results have also been reported for penta-BDE (commercial mixture) for gene mutation on *S. typhimurium* (TA98, TA100, TA1535, TA1537) and *S. cerevisiae*.

No relevant *in vivo* studies are available.

4.5 Carcinogenicity

In spite of an incomplete methodology (only 2 tested doses and consequently lack of NOAEL and no possibility of assessing a dose effect), the only studies to be taken into consideration are those undertaken by the NTP (1986). In Fischer rats that were administered deca-BDE (purity: 94-99%) at the dose of 1120 and 2240 mg/kg b.w./day (males) and 1200 and 2550 mg/kg b.w./day (females) for 103 weeks, the NTP reports an increased incidence of neoplastic hepatic nodules (in males and females) without a significant rise in carcinomas. In a similar study in B6C3F1 mice (3200 and 6650 mg/kg b.w./day for males and 3760 and 7780 mg/kg b.w./day for females), the

NTP reports an increased incidence of combined hepatic adenocarcinomas in males but carcinomas alone are not significantly higher. Hyperplastic follicular cells in the thyroid have been observed in males. Overall, these studies do not provide sufficient proof for deca-BDE to be considered carcinogenic. The International Agency for Research on Cancer has classified deca-BDE in group 3 (insufficient proof in humans and animals). Liver and thyroid tumours appear to fit into a context of enzyme induction that may be considered species specific.

No data are available for other congeners.

4.6 Study of reproductive and development functions

Detailed studies on the assessment of reproductive functions are lacking. There are no specific studies available on embryotoxicity, peri- and post-natality, or the impact on descendants (multi-generation study). Moreover, most studies have been undertaken on commercial mixtures with no information regarding the composition.

Concerning deca-BDE, a fertility study in rats exposed for 60 days, before mating, during gestation and lactation, to 100 mg/kg b.w./day of a commercial mixture (77.4% deca-, 22.8% octa- and 0.8% nona-BDE) showed no effects on the main reproduction parameters nor toxic effects on dams or their pups (Norris *et al.*, 1975). These results were confirmed by a development study conducted in accordance with the OECD guidelines with up to 1000 mg/kg b.w./day of a commercial mixture (97% deca-BDE) (Hardy *et al.*, 2002).

A decrease in sperm production and sperm count was observed in adult rats whose mothers had been treated with penta-BDE (BDE-99) via force feeding on the 6th day of gestation (60 or 300 mg/kg b.w.) with no effects on their fertility (Kuriyama *et al.,* 2005). However, the authors affirmed that sperm production in rats can be reduced by 90% without compromising fertility, which is not the case in humans.

However, more recent studies showed effects on the sexual development of rats whose mothers had been treated with penta-BDE (BDE-99) via sub-cutaneous injection (1 or 10 mg/kg b.w./day of penta-BDE) during gestation (G10 to G18) (Lilienthal *et al.*, 2006). An alteration of ovarian tissues in generation F1 from 60 µg/kg b.w. and bone anomalies in generation F2 have also been observed after single administration on the 6th day of gestation (Talsness *et al.*, 2005).

In rabbits, delayed ossification has also been observed at the maternotoxic dose of 15 mg/kg b.w./day of a mixture of octa-BDE (G7 to G19), but no effect at 5 mg/kg b.w./day (Breslin *et al.*, 1989).

4.7 Study of endocrine functions

Very recently, studies highlighted the effects of penta-BDE (BDE-99) on endocrine functions, as seen by a decrease in anogenital distance, concentrations of sex steroids, the number of ovarian follicles and thyroid weight and an increase in sugar preference in males (index of feminisation). These effects were observed in adult rats whose mothers had been treated with penta-BDE via sub-cutaneous injection (1 or 10 mg/kg b.w./day) during gestation (G10 to G18). The authors noted that these effects were detected in adulthood, i.e. long after the end of exposure to penta-BDE, thus demonstrating the persistence of effects (Lilienthal *et al.,* 2006).

In 2004, Stoker *et al.* had already reported a delay in sexual maturation in male and female rats, after exposure as of weaning and for 20 to 31 days to 60 mg/kg b.w./day of a commercial mixture of tetra- and penta-BDE (DE 71), which could be the result of effects on thyroid, as it is particularly sensitive to PBDEs.

Thus, after exposure to this same commercial mixture (DE 71) or to tetra-BDE (BDE-47), a decrease in T4 levels in rats and mice was reported on several occasions, with no effects on T3 and TSH levels or only in males. Histopathological thyroid modification has been observed from 60 mg/kg b.w./day after 20 to 31 days of exposure to DE-71 (Fowles *et al.*, 1994; Hallgren *et al.*,

2001; Zhou *et al*., 2002; Stoker *et al*., 2004). T4 levels also decrease in foetuses with exposed mothers, with a return to normal values 15 days after weaning, corresponding to the end of exposure from breast-feeding (Zhou *et al*., 2002).

The study of PBDEs' biological activity is merely beginning, with the initial results showing that some congeners are active *in vitro*. For example, BDE-19, -100, -155 and -49 are antagonists with close affinity for AR (androgen receptor) and PR (progesterone receptor) receptors and agonists with affinity for the ER alpha receptor (oestrogen receptor). They inhibit estradiol sulfotransferase activity and have potentiating effects on the proliferation of GH3 cells induced by T3 (Hamers *et al.,* 2006). Some hydroxylated PBDE metabolites (depending on their stereochemistry) could be endocrine disruptors (Meerts *et al*., 2000 and 2001).

One of the proposed mechanisms is a competition of PBDEs with T4 in ransthyretin receptors (TTR), the primary carrier protein of thyroid hormones in rats; however, its meaning for humans still needs to be determined (JECFA, 2005).

Furthermore, DE-71 showed anti-androgenic potential in the Hershberger assay in immature rats (Stoker *et al*., 2005).

In its 2005 summary, JECFA suggested that Ah receptor-dependent effects might be due to contamination of commercial mixtures with dioxin-like compounds.

To conclude, hormone changes have been reported with some PBDEs. Concerning the thyroid, while direct effects cannot systematically be extrapolated to humans, indirect effects caused by thyroid hormone changes can definitely affect reproductive functions and more specifically sexual maturation, embryo development and the neuro-behavioural activity of newborns.

4.8 Conclusions

The few studies that are available for assessing the toxic effects of PBDEs for a limited number of congeners (mainly penta-, octa- and deca-BDE), show that the liver, kidney and thyroid are the target organs affected by these molecules' toxicity. However, their proven inducing-potential sheds light on liver and thyroid toxicity in terms of extrapolation to humans. Some studies suggest that PBDEs could affect the nervous system and immune functions. Genotoxicity and carcinogenesis data are too limited to reach a definitive conclusion on these two points. Moreover, the results of various studies concur that PBDEs are potential endocrine disruptors.

In general, the data in the literature are too superficial or too old for a relevant analysis of these aspects due to the aforementioned lack of methodological precision. To date, no testing has been undertaken in non-rodents. Studies would need to be undertaken in accordance with internationally recognised protocols to examine the congeners that are the most frequently found in food and the environment and with a satisfactory degree of purity.

As a result, the results of available studies have not enabled us to determine a reference experimental toxicological dose that could be used to establish a tolerable daily intake.

5 DATA IN HUMANS: CONTAMINATION STUDIES

Although deca-BDE remains the only PBDE mixture whose use is authorised in the European Union, there are few results related to its occurrence in the environment or humans.

Published and current studies aiming to estimate PBDE levels in humans primarily examine PBDEs that are mostly found in tissues, i.e. tri-, tetra-, penta-, hexa- and hepta-BDE (# 28, 47, 99, 100, 153, 154, 183). The data are more limited for levels of exposure to octa-BDEs, nona-BDEs and deca-BDE. The same is true for their metabolites. In fact, analysis of deca-BDE (and to a lesser extent of congeners containing 8 and 9 bromine atoms) is difficult due to its high molecular weight and its very limited solubility, including in organic solvents.

No data related to contamination levels in the French population are available to date. However, a research programme (AFSSET, RD-2004-011) is currently in progress. The studies currently being undertaken in Europe are based on Dutch, Norwegian and Czech samples (FIRE programme). It has been established that tissue concentrations of PBDE in humans are, in general, higher in North America than in the rest of the world (Gill *et al.*, 2004).

Human contamination levels around the world have primarily been measured in adipose tissue, blood (plasma or serum) and breast milk.

Adipose tissue

The first studies were undertaken in the US using samples collected from 1980 to 1990, showing hexa-, hepta-, octa- and nona-BDE at respective mean values of 0.21; 0.18; 0.65 and 1 ng/g lipid (Stanley *et al.,* 1991). Deca-BDE was found only at very low levels and PBDEs with less than 6 bromine atoms were not assayed.

Since 1998, several studies have been undertaken to assess contamination of adipose tissue with PBDEs. The results are given in table 2.

Table 2: Concentrations of PBDEs found in adipose tissue

	No. of samples	PBDEs analysed	Concentration (ng/g lipid)	References
Belgium (2002)	20	28, 47, 99, 100,153	4.75 (2.2-11.7)	Covaci *et al.*, 2002
Belgium (2006)	53	28, 47, 99, 100,153, 154,183	11.1 (1.23-57.2)	Naert *et al.*, 2006
Spain (1999)	13	tetra-BDE, penta-BDE, hexa-BDE	1.36 0.93 1.83	Meneses *et al.*, 1999
Sweden (1995-97)	27	tetra-BDE	5.1 (0.6-27.5)	Hardell *et al.*, 1998
Japan (1970 and 2000)	10	28, 47, 99, 100,153, 154,183	**1970**: 0.029 (0.006-0.078) **2000**: 1.288 (0.466-2.753)	Choi *et al.* 2003
USA	23 F	47, 99, 100, 153,154	86	Shed *et al.*, 2002
USA (2003-2004)	52	brominated di-hexa	399 (17-9630)	Johnson-Restrepo *et al.,* 2005)
Sweden	1 F + 4 M	17, 28, 47, 66, 85,99, 100, 153,154	5.4 (3.8-7.7)	Meironyté-Guvenius *et al.*, 2001
Finland	10	47, 99,153	11.6 (5.5-22)	Strandman *et al.*, 1999

The primary PBDEs that were found, in terms of concentration, were BDE-47 (tetra), BDE-99 (penta) and BDE-153 (hexa) which together accounted for more than 85% of the detected PBDEs. BDE-47 was the principal congener in most of the studies.

These results were also observed by Antignac *et al.* (2006). These authors took samples from the adipose tissue of women giving birth via caesarean and measured concentrations ranging from 1.2 to 14.9 ng/g lipid (n=26; median value: 2.5 ng/g) for all tri- to hepta-BDEs, with BDE-153 as the dominant congener (around 50% of the total) followed by BDE-47 (around 25%) and other major congeners (BDE-183, BDE-99, BDE-100, BDE-28, BDE 154). Five congeners of octa-BDE, and the 3 nona-BDEs and deca-BDE, were also measured in these samples. The median values for these PBDEs were respectively 1.3; 0.8 and 2.3 ng/g lipid. These results clearly illustrate that currently available data could significantly underestimate the occurrence of congeners having a high molecular weight.

Blood

The first detailed study was undertaken in Sweden in 1997 (Klasson-Wehler *et al.,* 1997) in which 6 PBDEs, from tri- to hexa-BDE, were measured in blood (plasma). The results showed BDE-47 and BDE-99 as the dominant congeners (the two accounting for 70% of plasma PBDEs) for mean values of 2.1 ng/g lipid (all PBDEs combined).

Comparable studies (in terms of the tested PBDEs) were published for Germany, where total blood samples contained an average of 3.9 ng/g and 5.6 ng/g lipid of PBDEs, respectively in 1985 and 1999 (Schröter-Kermani *et al*., 1999, according to Gill *et al*., 2004). A study conducted in Norway among 40 to 50 men monitored between 1977 and 1998 showed that the PBDE (#28, 47, 99, 100, 153 and 154) contamination level had increased sevenfold, from 0.44 ng/g lipid in 1977 to 3.3 ng/g lipid in 1999 (Thomsen *et al.,* 2002).

Furthermore, data are available for Japan with median values of 4.1 ng/g lipid in whole blood (Hirai *et al*., 2002) and with BDE-47 and BDE-153 as the dominant congeners (the two accounting for 70% of the total).

In the United States, levels that were approximately 10 times higher were found for equivalent PBDEs, with, in particular, an average BDE-47 concentration of around 50 ng/g lipid (serum; state of Indiana; Mazdai *et al*., 2003). For BDE-47, in California, a very clear difference was found between archived samples dating from the 1960s (BDE-47 non-detectable) and those taken in the late 1990s (median 16.5 ng/g lipid) (Petreas *et al*., 2003). Söjdin *et al*. (2004) also showed an increase in BDE-47 levels between 1985-89 (5.4 ng/g lipid) and 2000-2002 (36 ng/g lipid). These various results clearly indicate the importance of anthropogenic sources in human contamination with PBDEs.

Sjodin *et al*. (2000) showed that there was a correlation between the rise in plasma levels measured for some PBDEs (particularly BDE-47) and a diet rich in fish, in samples from Sweden and Lithuania, for people consuming fish from the Baltic Sea.

Breast milk

Studies of archived samples of breast milk suggest an increase in residual levels of organobromines in humans, concurrent with the increased production of brominated flame retardants over the last few decades. The results give median levels ranging from less than 0.1 ng/g lipid for the oldest studies (1970s) to 196 ng/g lipid in a 2001 study conducted in the United States. Most of the other studies found median levels of less than 10 ng/g lipid in breast milk.

The studies undertaken by Noren and Meironyté (1998, 2000) and Meironyté *et al.* (1999, 2001) on Swedish samples showed an increase in levels measured over time, ranging from 0.07 ng/g lipid in 1972 to 4.02 ng/g lipid in 1997 for BDE-28, 47, 66, 85, 99, 100, 153 and 154. BDE-47 (>50% of the total) and, to a lesser extent, BDE-99, -100 and -153 were found to be the dominant congeners. The samples, from a storage bank of human milk, were made of mixtures with equivalent quantities of milk stored in 1972 (n=75), 1976 (n=78), 1980 (n=116), 1984 (n=102), 1985, 1990, 1994, 1996 (n=20) and 1997 (n=40) (Meironyté *et al*., 1999). Based on this, the authors concluded, for the 8 assayed PBDEs combined, that measured values had increased exponentially, doubling every 5 years. The results of the retrospective study undertaken by Fängstrom *et al*. (2005) on the Faroe Islands examining milk mixtures from 1987, 1994-95 and 1999 confirmed a sharp increase in measured levels (BDE-47, 99, 100, 153, 209). In this study, the calculated averages for all of the tested PBDEs were respectively 1.9; 4; and 8 ng/g lipid. Deca-BDE, which was not measured in the Swedish studies, accounted for a non-negligible proportion of the total (around 10%). Moreover, on the Faroe Islands, BDE-153 was found in larger quantities than BDE-47.

Ohta *et al*. (2002) assayed PBDEs in the milk of women whose diets they determined. They concluded that for all of the 6 PBDEs from tri- to hexa-BDE, the levels measured in milk were higher in women with a diet rich in fish (1.7 ng/g lipid) than those of women who seldom ate fish (0.8 ng/g lipid).

In the United Kingdom, Kalantzi *et al*. (2004) analysed 15 PBDEs in milk samples collected from 54 women between 2001 and 2003. The measured concentrations ranged from 0.3 to 69 ng/g lipid (geometric mean: 6.9 ng/g lipid). Comparable results were obtained in Sweden by Darnerud *et al.* (2003) with 3.8 ng/g lipid and in Poland by Jaraczewska *et al*. (2006) with 2.0 ng/g lipid.

In all of the aforementioned studies, BDE-47 was usually dominant, followed by BDE-153. The prevalence of BDE-153 in the milk of women from the Faroe Islands could be related to their diet

which includes the meat of marine mammals. It should be noted that current studies (FIRE programme, Prague meeting 2005, unpublished) tend to indicate that measured values have been stabilising in Europe over the past few years.

Pregnancy and breastfeeding

Some studies that have been undertaken in humans suggest that some PBDEs cross through the placenta.

In Finland, BDE-28, -47, -99 and -153, assayed in human placentas, had concentrations between 1 and 4.4 ng/g lipid (Strandman *et al*., 2000).

Guvenius *et al*. (2003) sampled the maternal blood, umbilical cord blood and breast milk of 15 Swedish women and analysed the concentration of 10 PBDEs in these samples. They found PBDE concentrations of 2.07 ng/g lipid in maternal blood, 1.69 ng/g lipid in umbilical cord blood and 2.14 ng/g lipid in breast milk. However, compared with fresh weight, concentrations in umbilical cord blood were 5 times lower than in maternal blood.

In a study of 12 women undertaken in the United States (Mazdai *et al.,* 2003), the concentrations of 6 PBDEs measured in maternal blood and the umbilical cord were respectively 15 to 580 ng/g lipid and 14 to 460 ng/g lipid, or around 7 to 230 times higher than in the Swedish study (Guvenius *et al*., 2003). In both studies, BDE-47 was dominant. A similar study was undertaken by Bi *et al.* (2006, People's Republic of China, 21 foetal serum/maternal serum combined samples), in which a median value of 3.9 ng/g lipid was determined for the 7 PBDEs (BDE-28, -47, -99, -100, -153, -154, -183) tested in the blood of the umbilical cord. In this study, BDE-47 and -153 were dominant (the two accounting for 60% of the total). Mazdai *et al*. (2003) also assayed total and free T3 and T4 thyroid hormones in maternal blood and the umbilical cord. They did not find a relationship between the total level of PBDEs and concentrations of T3 and T4, unlike what has been observed in mice and rats exposed to PBDEs (Hallgren *et al.,* 2001).

The transfer of PBDEs across the human placenta was also strongly suggested by the studies of Schecter *et al.* (2005), who measured PBDE levels ranging from 4 to 33 ng/g lipid in 10 livers of stillborn children and children who died shortly after birth.

6 LEVELS OF CONTAMINATION IN THE ENVIRONMENT

Levels of PBDE residues were measured for various fish species, and were found to be as high as 27 µg/g lipid in pike muscle, in animals exposed to industrial textile effluents (Andersson *et al*., 1981). High levels (up to 28 µg/g lipid) have also been measured in the eggs and liver of marine birds (Sellstrom *et al*., 1993) and in cetaceans (0.8-3.1 µg/g lipid) (Lindstrom *et al*., 1999). It should be noted that many of these studies refer to marine animals (or predators of marine animals) in the Baltic Sea region, where there is substantial contamination with organic halogen compounds. Likewise, the most recent studies (2000-2005) largely come from North America where, on the one hand, environmental contamination levels are higher than in Europe (same as human contamination levels) and, on the other hand, penta- and octa-BDE mixtures are still used. The BFR levels found in various biological environments and samples were the subject of a detailed review in 2006 (Law *et al*.). The most recent results highlight concentrations of deca-BDE (as well as other BFRs: HBCD and TBBPA).

Aside from these studies on PBDE levels detected in wild fauna, a few publications provide information on possible food sources of PBDE. A 2000 study conducted in Japan on samples from 7 fish species (Akutsu *et al*., 2003) showed a strong majority of BDE-47 all species combined, with residual levels ranging from 1 to 38 ng/g lipid (0.06 to 2.1 ng/g fresh weight). PBDEs with neighbouring molecular weights (tri- and penta-BDE) were also detected. These authors included the analysis of deca-BDE in addition to congeners up to hexa-BDE, but the latter was found only in two fish species at marginal doses.

A less extensive list of PBDEs (tetra- to hexa-BDE) was tested in freshwater fish in the United States (Hale *et al*., 2001). The measured levels ranged from 5 to 47900 ng/g lipid, with a strong majority of BDE-47, -99 and -100. However, in the study area (Virginia), several industrial sources

could explain such results. Many other studies indicate that freshwater fish and marine fish are major sources of PBDE contamination for humans.

7 ESTIMATE OF PBDE CONTAMINATION LEVELS IN FOODS

Recent studies have indicated residual levels of PBDE in a wide range of food products. The main studies have been conducted in Japan (Ohta, 2002), Spain (Bocio *et al.,* 2003), Finland (Kiviranta *et al*., 2004) and the United States (Schecter *et al*., 2004). The results of PBDE analyses, undertaken by the Canadian, Dutch and German authorities, are given in the JECFA report (2005). In France, the *Calipso* study (AFSSA/DGAL/INRA, 2006), which examined local seafood sourcing methods of large consumers', estimated levels of PBDE contamination in these products.

It should be noted that the quantity and quality of available data vary greatly by country. The study methodology is not always described and the number of assayed congeners is not always indicated. When a congener is not quantified, its contamination is considered to be nil or equal to the limit of detection (LOD) or the limit of quantitation (LOQ), which can cause contamination to be underestimated or overestimated. Data processing (aggregation of samples or individual data) and the expression of results can also differ. In general, the fact that not all of these parameters are described and the methodological differences make it difficult to compare results.

In the Japanese study (Ohta, 2002), 11 PBDEs were tested, from tri- to hexa-BDE. The highest levels were measured in fish with, for some species, more than 1.5 ng of PBDE per gram of fresh weight (ng/g FW) (combination of 6 PBDEs, from tri- to hexa). The plant products studied – spinach, potatoes and carrots – respectively contained 0.134, 0.048 and 0.003 ng/g FW and pork, beef and chicken meat respectively contained 0.064, 0.016 and 0.006 ng/g FW. BDE-47 was the main congener in most cases, but other PBDEs were also found, and were even dominant in some vegetable and meat samples (hexa-BDE: potatoes and carrots; penta-BDE: pork). The values measured for vegetables were high compared with Spanish data.

Bocio *et al*. (2003) analysed PBDEs (from tetra-BDE to octa-BDE) in 54 composite samples taken in 2000 in retail stores and at markets in Catalonia ('shopping basket' study). The average contamination levels found in foods were as follows: 0.334 ng/g FW for fish and shellfish; 0.588 ng/g FW for oil and fat; 0.109 ng/g FW for meats and by-products; 0.065 ng/g FW for eggs; 0.048 ng/g FW for dairy products; 0.017 ng/g FW for milk; 0.036 ng/g FW for cereals; 0.011 ng/g FW for dried vegetables; 0.006-0.008 ng/g FW for fruit and vegetables. One of this study's major advantages was to show that the distribution of the various PBDEs is not the same according to the commodity used For example, while tetra- and penta-BDEs are the main congeners for seafood (0.158 and 0.115 ng/g FW for tetra- and penta-BDE), the distribution of PBDEs is much more homogeneous in other foodstuffs, and particularly in meat products (around 0.023 ng/g FW for each analysed PBDE).

Kiviranta *et al*. (2004), in a 'shopping basket' study, analysed BDEs # 47, 99, 100, 153 and 154 in 4,000 samples corresponding to 228 foods collected from 1997 to 1999 in retail stores and at markets in Finland. The concentrations of these 5 PBDEs ranged from 0.00082 ng/g FW (liquid dairy products) to 0,850 ng/g FW (fish). Concentrations in plant products (cereal products, potato-based products, vegetables and fruit) ranged from 0.0013 to 0.017 ng/g FW.

Schecter *et al*. (2004) conducted a 'shopping basket' study of 32 food samples (animal products, fish and dairy products) collected from 3 supermarkets (Dallas, USA) in which they assayed 13 PBDE congeners including deca-BDE. The median values obtained were 1.725 ng/g FW for fish (BDE-47 dominant, then BDE-99 and BDE-100), 0.283 ng/g FW for meat and 0.032 ng/g for dairy products (BDE-47 dominant, then BDE-99). It is important to note that, while this study was presented as preliminary, the data provided showed high proportions of deca-BDE in some fish and in other samples (veal liver, cheese). Moreover, the PBDE residue levels detected in skimmed milk in this study were lower than the limit of detection.

In 2001, the United Kingdom (UK-COT, 2004) tested for BDE # 28, 47, 99, 100, 153 and 154 in trout and eel samples taken from the Skerne and Tees rivers that were home to an industrial chemical complex. This study showed that PBDE concentrations in trout varied from 12-14 ng/g FW upstream to 59-197 ng/g FW downstream and for eel from 53 ng/g FW upstream to 164-288 ng/g FW downstream from the industrial site. In another broader study, the United Kingdom (UK, 2006a) analysed 17 BDEs in 24 wild fish species, 7 farmed fish species, 7 molluscs and shellfish and 10 processed or tinned fish and mollusc and shellfish products at a rate of 30 to 60 samples per species and in 10 food supplements made with fish oil, sampled on the British market from 2002 to 2004. BDE 47 was dominant; BDEs # 49, 99, 100, 153 and 154 were usually detected. BDE 209, when found, was detected at high concentrations. The observed levels in farmed trout and eel were generally lower than those found in the 2001 study. In food supplements made with fish oil, concentrations of BDE 28 and 153 were 8 times higher than in fresh and tinned fish. The United Kingdom also analysed these 17 BDEs in 17 food groups. The meat group had the highest concentrations of PBDEs, dominated by BDE 209 (3.64 ng/g fresh weight). In the other groups, BDE 209 was also the dominant congener with concentrations ranging from less than 0.006 to 0.39 ng/g FW (UK, 2006b).

In 2002, for a total diet study (TDS), the United Kingdom (UK-COT, 2004) also assayed the 6 PBDEs in foods sampled at 24 different sites. The results were lower than the limit of detection.

From 2001 to 2003, Germany (JECFA, 2005) assessed the contamination of various foodstuffs with BDEs # 28, 47, 99, 100, 153 and 154 with a limit of quantitation (LOQ) of 1 ng/g fat. Concentrations ranged from 1 to 5 ng/g fat in eggs (30 samples out of 106 > LOQ), from 1 to 12 ng/g fat in chickens (14 out of 38 samples), from 1 to 4 ng/g fat in milk (5 out of 96 samples), and from 1 to 16 ng/g fat in pork (10 out of 48). In another study, Päpke *et al* (2004) showed that the fish with the highest contamination levels were pike (max: 47.6 ng/g fat) then herring (13.9 ng/g fat) and halibut (0.42-9.86 ng/g fat) (total of BDEs -17, -28, -47, -66, -77, -99, -100, -153, -154, -183 and -209).

The Netherlands (de Winter-Sorkia *et al*, 2003) tested for BDEs # 28, 47, 71, 77, 99, 100, 153, 154, 190 and 209 in 91 foodstuffs consumed by the Dutch population. BDEs # 71, 77, 190 and 209 were never detected (LOD: 0.5 ng/g). The concentrations measured in cheese, pork, poultry and beef ranged from 0.3 to 2.1 ng/g. Herring was the most contaminated foodstuff with an average concentration of 12.9 ng/g and then salmon with 3.4 ng/g. In milk, other dairy products and animal and plant fats, PBDE levels were lower than LOD.

JECFA (2005), referring to the available contamination data in Europe, estimated contamination levels for 6 PBDEs (# 28, 47, 99, 100, 153 and 154) in the various food groups (table 3). Fish and seafood were some of the most contaminated foods with an average PBDE concentration of 1.8 ng/g fresh weight.

Table 3: Parameters of distribution of foodstuff contamination for 6 PBDEs (# 28, 47, 99, 100, 153 and 154) in Europe (ng/g fresh weight)
(*Source: JECFA data, 2005*)

	Parameter	Dairy products	Eggs	Meat and poultry	Fruit and vegetables	Fats and oils	Fish and seafood
Scenario 1 ND = 0	Mean	0.030	0.048	0.078	0.007	0.267	1.782
	Median	0.023	0.037	0.060	0.005	0.204	1.364
	P90	0.055	0.090	0.146	0.013	0.496	3.316
Scenario 2 ND=LOD	Mean	0.249	0.128	0.232	0.010	0.944	1.872
	Median	0.190	0.098	0.178	0.007	0.723	1.433

	P90	0.463	0.239	0.433	0.018	1.757	3.484

The *Calipso* study (AFSSA/DGAL/INRA, 2006) analysed 7 PBDEs (# 28, 47, 99, 100, 153, 154, 183) in 170 fresh and deep-frozen, tinned, smoked and ready-made seafood products sampled at the 4 sites chosen for this study (Le Havre, Lorient, La Rochelle and Toulon). The following were taken into account to make the sampling representative:

- results of consumption frequencies and quantities consumed obtained for the consumption survey,
- sourcing methods (fresh, semi-fresh, frozen, tinned products, etc.),
- sourcing sites (seashore fishing, purchase at harbour, at the market, from a fish merchant, in another type of store or consumption away from home),
- product origins (preferably local, regional, etc.).

One hundred and ten fish samples (representing 31 species) and 44 mollusc and shellfish samples (representing 17 species) were analysed. The results are given in table 4.

Table 4: Parameters of distribution of seafood contamination
for 7 PBDEs (# 28, 47, 99, 100, 153, 154 and 183) in France (ng/g fresh weight) (*Calipso* study)

	Fish	**Molluscs and shellfish**
Mean	1.739	0.562
Standard deviation	4.670	0.673
Median	0.586	0.323
P90	2.393	0.863
P95	2.643	1.427

Similar concentrations have been observed in France as by JECFA (table 3) for all of Europe. The most contaminated fish is eel with an average concentration of 26.6 ng/g FW. Tinned pilchard, mackerel and smoked salmon have respective average concentrations of 3.2, 2.8 and 2.7 ng/g FW. For molluscs and shellfish, spider crab is the most contaminated species with 3 ng/g FW. Dogfish for fish and octopus and scallops for molluscs and shellfish are the least contaminated species (0.3 and 0.2 ng/g FW). In general, PBDE concentrations in seafood increase with fat content. BDE-47 and BDE-99 are found the most frequently in seafood, respectively accounting for around 60% and 23% of total contamination for the 7 PBDEs.

8 ESTIMATE OF CONSUMER EXPOSURE TO PBDES

8.1 Exposure in various countries in Europe, North America and Asia

Table 5 shows that total diet studies (TDS) published by several countries – Canada and the United States for North America; Finland, Netherlands, Spain, Sweden and United Kingdom for Europe; and Japan for Asia – give daily mean exposure levels from 13 to 228 ng PBDE per person.

Table 5: Average exposure to PBDEs in various countries (ng/person/day)
(Source: JECFA data, 2005)

Countries	**Study types**	**Analysed PBDEs**	**Average exposure to PBDEs (ng/person/day)**

Canada	TDS, 50 samples	28, 47, 99, 100, 153, 154, 183	30 to 44
Spain	TDS, 54 samples	47, 99, 100, 153, 154, 183, octaBDE	82-97
Finland	TDS, 228 samples	47, 99, 100, 153, 154	43
Japan	TDS, 13 samples	47, 49, 66, 99, 100, 119, 153, 154, 183	113
Netherlands	TDS, 84 samples	28, 47, 71, 77, 99, 100, 153, 154, 190, 209	13-228
Sweden	TDS, 20 samples	47, 99, 100, 153, 154	41 to 52
USA	TDS, 32 samples	17, 28, 47, 66, 85, 99, 100, 138, 153, 154, 183, 209	100 to 140

Table 6 gives the contributions of major food groups to exposure to PBDEs found in Europe and North America (JECFA, 2005). In Europe, fish are the main contributor to dietary exposure to PBDEs whereas in North America, the main contributors are land-based animal products (dairy products, meat and poultry).

Table 6: Contribution of various dietary vectors to adult consumers' daily exposure to PDBEs (expressed in ng/person/day and %) in Western Europe and North America (*Source: JECFA data, 2005*)

Scenario	Diet	Dairy products		Eggs		Meat and poultry		Fish and seafood		Fruit and vegetables		Fats and oils		Total
		ng/day	%	ng/day	%	ng/day	%	ng/day	%	ng/day	%	ng/day	%	ng/day
Scenario 1 ND=0	Western Europe	10	*8*	2	*1*	17	*13*	81	*64*	6	*4*	13	*10*	128
	North America	25	*13*	8	*4*	63	*33*	42	*22*	6	*3*	46	*24*	188
Scenario 2 ND=LOD	Western Europe	81	*30*	5	*2*	49	*18*	85	*31*	8	*3*	45	*17*	274
	North America	81	*34*	5	*2*	59	*22*	42	*17*	8	*3*	52	*22*	240

8.2 Estimate of exposure in France

The *Calipso* study (AFSSA/DGAL/INRA, 2006) includes a consumption survey that targeted large consumers of fish and seafood in 4 French coastal regions (Le Havre, Lorient, La Rochelle and Toulon). Sampling representativeness of the interviewed population was ensured via random recruitment. One thousand and one individuals, or 250 per site, were included in this study, and met the following criteria:

- adult population (18 years or more),
- consumed seafood at least twice a week, a criterion defined from the 1999 INCA study. The median consumption frequency calculated based on individual data on seafood consumption in the INCA 1999 survey's population was twice a week (INCA, 2000),
- had continuously lived at one of the selected sites for a given number of years.

Estimate of exposure taking into account all PBDE vectors

Exposure to PBDEs was estimated based on the data on seafood contamination measured in the *Calipso* study (# 28, 47, 99, 100, 153, 154, 183) and European data on contamination in other groups of PBDE vectors (table 3) according to a low hypothesis (nd=0) and a high hypothesis (nd=LOD) applying two deterministic exposure scenarios:

- the 1st scenario was based on the consumption data from the 1999 INCA survey (adult and child populations);

- the 2nd scenario used the consumption data of the *Calipso* survey that examined fish consumption data only, considering that on average, the *Calipso* population sample consumed other PBDE vectors at levels similar to those consumed by the INCA adult population sample.

The results of these two scenarios are given in table 7.

Table 7: Estimate of exposure to PBDEs (ng/person/day) in the adult and child French population from the INCA survey and large consumers of seafood from the *Calipso* study

Food group	Exposure in the French population (1999 INCA survey)				Exposure of large consumers of seafood in France (*Calipso* study)	
	child (3 to 14 years, n = 1018)		adult (15 years and over, n = 1474)		adult (18 years and over, n = 1011)	
	Mean (low-high)	% contribution (low-high)	Mean (low-high)	% contribution (low-high)	Mean (low-high)	% contribution (low-high)
Milk and dairy products	9.9-82	19.7-60.7	7.0 - 58	11-41	7.0-58	4.1-23
Eggs	0.6-1.5	1.2-1.0	0.86-2.3	1.4-1.6	0.86-2.3	0.5-0.9
Meat and poultry	5.7-17	11.4-12.5	7.8-23.2	12.3-16.4	7.8-23.2	4.5-9.3
Fish and seafood	28.2-28.2	56.6-21	41.6-41.6	66-29	150-150	87-60
Fruit and vegetables	1.6-2.3	3.2-1.6	2.3-3.3	3.6-2.3	2.3-3.3	1.3-1.3
Fats and oils	4.0-4.0	8-3.2	3.7-13.2	5.9-9.3	3.7-13.2	2.2-5.3
Total	**50-135**	**100**	**63-142**	**100**	**172-250**	**100**

These results show that mean exposure to PBDEs is:
- for children (INCA survey), 50 to 135 ng/person/day (or 2.5 to 7.2 ng/kg b.w./day for an average weight of 20 kg);
- for adults (INCA survey), 63 to 142 ng/person/day (or 1.0 to 2.2 ng/kg b.w./day for an average weight of 65 kg);
- for adult large consumers of seafood (*Calipso* survey), 172 to 250 ng/person/day (or 2.5 to 3.7 ng/kg b.w./day for an average weight of 68 kg). On average, this population consumed four times more fish and seafood than the adult French population from the INCA survey.

The major vectors that contribute to French exposure are, in decreasing order: fish and seafood (21 to 87%), milk and dairy products (4 to 60%), meat and poultry (4 to 16%), and fats and oils (2 to 10%). Other vectors contribute at levels lower than 5% of total exposure. This contribution is comparable to that observed in other European countries, particularly for the main vector represented by fish and seafood, which is 30 to 60% of total exposure (see table 6).

Estimate of exposure in large consumers of seafood through these foods alone

Table 8 gives the estimated exposure among the *Calipso* survey population to PBDEs, via their consumption of fish and seafood.

Table 8: Exposure of large consumers of seafood to the 7 PBDEs via seafood (*Calipso* study)

Population group	n	Exposure	
		ng/person/day	ng/kg b.w./day
Adult men (18-64 years)	246	155.3	2.1
Adult women (18-64 years)	641	145.7	2.4
Elderly subjects (65 years and over)	124	160.5	2.3
Women of childbearing age (18-44 years)	350	139.0	2.3
Total	1011	150.0	2.2

This estimate varies from 2.1 to 2.4 ng/kg b.w./day according to age group and sex. There is little difference between the groups as few differences were observed in terms of product contamination and because the various population groups had similar consumption levels.

Table 9 shows that the fish and seafood species, in all sourcing forms (fresh, deep-frozen, tinned and/or smoked), that contribute to 5% or more of total exposure to PBDEs are salmon, mackerel, sardines, cod, sea bass, pollock and tuna.

Table 9: Contribution of major 'fish and seafood' vectors to PBDE exposure via the consumption of fish and seafood (*Calipso* study)

Species	Contribution to exposure (%)	Average contamination (ng/g FW)	Average consumption (g/person/week)
Salmon (fresh, smoked)	18.5	2.6	74
Mackerel (fresh, tinned, smoked)	8.4	2.7	43
Sardines (fresh, tinned)	6.4	2.1	38
Cod	6.0	0.5	95
Sea bass	5.5	2.4	25
Pollock	4.9	0.7	57
Tuna (fresh, tinned)	4.9	0.6	75

In the case of salmon, the quantity consumed is the major factor among the contributors. For this fish, the highest contributor at up to 18.5%, similar PBDE concentrations were observed between composite samples of fresh/deep-frozen and smoked salmon, i.e. around 2.6-2.7 ng/g FW. However, because of the sampling method used, it is not possible to determine the respective share of wild salmon versus farmed salmon. Hites *et al.* (2004) estimated that farmed salmon would have significantly higher levels of PBDEs than wild salmon, with Chinook being the most contaminated wild species.

9 ANALYSIS METHODS

The vast majority of analytical methods developed for brominated flame retardants (BFRs) are relatively similar to those used for other types of lipophilic halogenated contaminants such as dioxins and PCBs. The specific analysis of BFRs however is somewhat unique and requires specific laboratory expertise. The wide range of substances concerned, including PBDEs as well as tetra-bromo-bisphenol-A (TBBP-A) and hexa-bromo-cyclododecane (HBCD), complicates the development of multi-residue methods due to the heterogeneous physicochemical properties of these various families. Their ubiquitous character, i.e. their omnipresence in the environment and in many manufactured products, including some laboratory consumables and equipment, makes it extremely difficult to manage the analysis of contamination and sometimes to interpret the results.

Any sample (food matrix or sample of human origin) should be protected as soon as possible, and even before laboratory extraction, by storing it in a deep-freezer (-20°C) and away from light, to prevent the potential degradation of PBDEs and particularly their debromination. Plastic materials (tubes, etc.) must not be used for sampling and storage. Regardless of which option is chosen, possible interference between the container and the analysis result should be assessed for each type of container. The best options could be the use of glass bottles and tubes (liquid samples) and aluminium foil placed in boxes for bulky solid samples.

The first stage of sample treatment generally consists in extracting the fat in the samples, using liquid/solid, cold liquid/liquid, accelerated solvent (ASE) or Soxhlet extraction. PBDEs, like dioxins and PCBs, are highly lipophilic compounds (their octanol/water partition ratio or K_{ow} is generally 5 to 9), which promotes their bioaccumulation in fatty tissues. Concentration measurements are generally expressed according to fat content, although this regulatory aspect has not yet been definitively established for BFRs. Extracts must then be purified. Multi-layer filled columns of silica activated with sulphuric acid, alumina and/or florisil, derived from the methods used for dioxins and furans, are widely used in this field. A last analytical challenge related to purification lies in the separation of various types of BFRs (PBDE, TBBP-A and HBCD), in order to have separate classes of BFRs which can be measured with an appropriate technique.

As far as measurement strictly speaking is concerned, chromatography-mass spectrometry is clearly the best method for identifying and quantifying these compounds at very low concentration levels. While gas chromatography (GC) is the reference technique used for separating various PBDE congeners, liquid chromatography (LC) is currently necessary for analysing HBCD, as this technique is presently the only one that can separate the three isomers (α, β and γ). TBBP-A analysis may be considered either using GC (after derivation) or LC (without derivation), with the former technique remaining the most sensitive. Note that recent technological innovations are perhaps in the process of changing the situation with respect to liquid chromatography, particularly due to the development of ultra-resolvent systems and new interfaces including photoionisation (APPI), an ionisation technique whose principle is closely tailored to apolar compounds, including PBDEs.

After they have been separated, compounds of interest can be measured using various techniques, with different performances in terms of sensitivity and specificity. Electron capture detection (ECD) was historically one of the first techniques used to measure organic halogen compounds, including PBDEs. The highly electrophilic character of bromine atoms fosters electron capture reactions and makes this detection technique very sensitive. Although still occasionally used, it is limited due to its low degree of specificity, as the measured signals cannot identify the exact original structure of the assayed compounds. On the other hand, mass spectrometry (MS) is both highly sensitive, achieving the expected residual levels, and highly specific, enabling the unambiguous identification of the tested compounds.

Electron impact (EI) ionisation generally produces a sufficient number of diagnostic ions to confirm the compounds' identity. These correspond either to molecular ions or to losses of one or more bromine atoms. Negative chemical ionisation (NCI) is another ionisation method that is frequently applied to PBDEs. This ionisation method promotes the formation of bromine's characteristic ions (m/z 79 and 81), which can therefore be monitored with excellent sensitivity. However, this technique is less specific than electron impact ionisation, due to a lack of additional diagnostic signals enabling the accurate identification of compounds. Moreover, the sole monitoring of Br ion signals means that internal standards labelled [^{13}C] cannot be used for quantitation, since in these conditions, their signals overlap those of native compounds [^{12}C]. Nevertheless the utility of [^{13}C]-labelled standards in guaranteeing excellent quantitative performance has been proven.

In comparison with one-dimensional mass spectrometry (MS), tandem mass spectrometry (MS/MS) generally improves the specificity of diagnostic signals, and consequently lowers detection levels. This technique has been used by several authors on the basis of triple quadrupole or ion trap systems. In general, the detection levels achieved using the latest-generation devices appear to be compatible with expected residue levels in foodstuffs. However, high-resolution mass spectrometry (HRMS) still remains the technique of choice in terms of sensitivity and specificity. Because bromine atoms are present on the molecules in question, these have a 'mass defect' which can be exploited in high-resolution mass spectrometry, and as a result, almost all interfering signals can be eliminated. Furthermore, this type of equipment is consistent with a joint approach to the measurement of dioxins, furans and PCB.

The most commonly used methods for the extraction/purification of BFRs from food matrices mainly use Soxhlet extraction, liquid/liquid extraction and silica extraction columns. The management of external contaminants remains a major problem for some indicator PBDEs that may require special infrastructures and drastic quality control. The most frequently used detection methods still remain GC-NCI-MS and GC-EI-HRMS, with the latter being the method of choice, consistent with a joint approach to the control of dioxins, furans and PCB. Nevertheless, some difficulties persist for the specific analysis of deca-BDE, which is particularly sensitive to degradation. LC-MS/MS and GC-MS/MS probably represent long-term alternatives to GC-HRMS.

10 CONCLUSIONS AND RECOMMENDATIONS

In light of current knowledge of PBDEs, the French Food Safety Agency (AFSSA) has reached the following conclusions and made the following recommendations.

1 Deca-BDE is the most commonly used PBDE worldwide and the only PBDE authorised for use in the European Union. Because of its physicochemical properties, this compound is considered to be much less bioaccumulable and bioavailable than lighter congeners such as tetra-BDE. However, the degradation of deca-BDE under the effect of UV radiation is capable of producing all of the less brominated PBDEs.

2 There are relatively few studies of the toxicokinetics of PBDEs and these concern only a limited number of PBDEs, mainly deca-BDE and some penta- and tetra-BDEs including BDE-47. The persistence of BDE-209, particularly in adipose tissue, is relatively low, whereas that of BDE-47 and -153 is higher. These results, although incomplete, concur with the fact that these two BDEs (and by extension, penta- and hexa-BDEs) are dominant in fatty foodstuffs and particularly foodstuffs of animal origin (meat and fish).

AFSSA, considering that it is essential to be able to document the fate and metabolism of PBDEs in the body in order to assess risks related to these molecules, recommends encouraging the implementation of toxicokinetics studies undertaken in accordance with internationally recognised testing protocols.

3 Toxicological data are deemed insufficient to define relevant toxicological reference values.

AFSSA recommends implementing toxicity studies in accordance with internationally recognised testing protocols to assess the effects of PBDEs by using isolated congeners (excluding mixtures) that have closely controlled impurity profiles. Such studies should firstly be conducted on the PBDEs that are the most frequently found in humans.

4 Concerning dietary exposure, the main food vectors are, in order of importance: fish and other seafood, milk and dairy products, then meat.

In light of the available data on the seafood category (see *Calipso* study), AFSSA recommends preferably focusing PBDE testing on other products that are likely to significantly contribute to exposure such as meat, poultry and milk and dairy products for which no data are available in France.

5 The analysis of PBDEs principally found in matrices of human origin and in food products, i.e. tri-, tetra-, penta-, hexa- and hepta-BDEs (# 28, 47, 99, 100, 153, 154, 183), and particularly tetra- and penta-BDEs, remains a priority: The case of PBDEs with high molecular weights, and especially BDE-209, should not be neglected. So far, few studies have provided data on these PBDEs as to their occurrence in human tissues and food matrices.

AFSSA recommends assaying the following PBDEs: # 28, 47, 99, 100, 153, 154, 183 and 209. Moreover, given that in the future, it may be necessary to collect data on other brominated flame retardants, AFSSA concurs with the EFSA opinion and recommends testing as well for HBCD, TBBPA and PBB 153.

6 GC-MS (GC-NCI-MS or GC-EI-HRMS) is presently the most appropriate method for PBDE assaying in human and food matrices. There are two major problems that make it difficult to accurately assess PBDE levels in these matrices:

- the possible contamination of samples at practically all stages of analyte preparation (sampling, extraction, environment and laboratory equipment);
- the technical difficulty involved in assaying the heaviest compounds, and particularly deca-BDE.

As a result, only laboratories specialising in the assaying of this type of organic halogen compound, having duly validated their sample preparation and analysis methods, are capable of producing reliable results.

AFSSA draws attention to difficulties related to PBDE assaying and recommends ensuring that the laboratories responsible for analysing PBDEs have taken all necessary measures to guarantee the reliability of their results.

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Annex 1

Chemical structures of the main brominated flame retardants (BFRs)

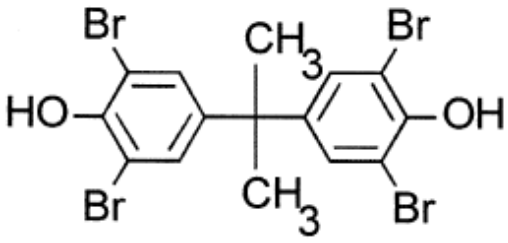

Figure 1: Structure of tetra-bromo-bisphenol A (TBBPA)

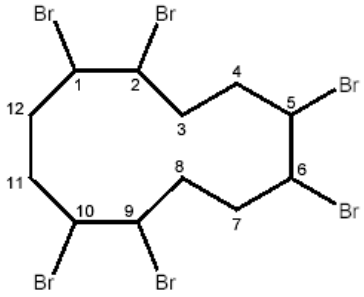

Figure 2: Structure of hexa-bromo-cyclododecane (HBCD)

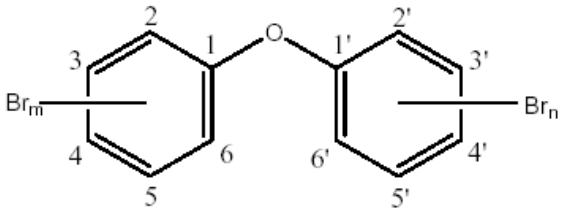

Figure 3: Structure of polybrominated diphenyl ethers or PBDEs.
This family has 209 possible congeners,
defined by the number of bromine atoms per molecule

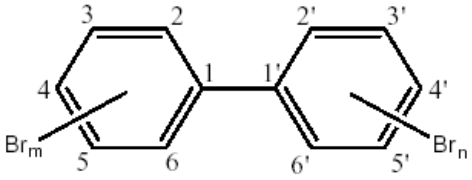

Figure 4: Structure of polybrominated biphenyls or PBBs.
This family has 209 possible congeners,
defined by the number of bromine atoms per molecule

ANNEX 2

IUPAC numbering of the 209 PBDE congeners

IUPAC number (left) and numbers of substituted carbons (right). Underlined are the most commonly tested PBDEs which are most often found in food matrices and biological environments.

monoBDE	
1	2
2	3
3	4

diBDE	
4	2,2'
5	2,3
6	2,3'
7	2,4
8	2,4'
9	2,5
10	2,6
11	3,3'
12	3,4
13	3,4'
14	3,5
15	4,4'

triBDE	
16	2,2',3
17	2,2',4
18	2,2',5
19	2,2',6
20	2,3,3'
21	2,3,4
22	2,3,4'
23	2,3,5
24	2,3,6
25	2,3',4
26	2,3',5
27	2,3',6
28	**2,4,4'**
29	2,4,5
30	2,4,6
31	2,4',5
32	2,4',6
33	2,3',4'
34	2,3',5'
35	3,3',4
36	3,3',5
37	3,4,4'
38	3,4,5
39	3,4',5

tetraBDE	
40	2,2',3,3'
41	2,2',3,4
42	2,2',3,4'
43	2,2',3,5
44	2,2',3,5'
45	2,2',3,6
46	2,2',3,6'
47	**2,2',4,4'**
48	2,2',4,5
49	2,2',4,5'
50	2,2',4,6
51	2,2',4,6'
52	2,2',5,5'
53	2,2',5,6'
54	2,2',6,6'
55	2,3,3',4
56	2,3,3',4'
57	2,3,3',5
58	2,3,3',5'
59	2,3,3',6
60	2,3,4,4'
61	2,3,4,5
62	2,3,4,6
63	2,3,4',5
64	2,3,4',6
65	2,3,5,6
66	2,3',4,4'
67	2,3',4,5
68	2,3',4,5'
69	2,3',4,6
70	2,3',4',5
71	2,3',4',6
72	2,3',5,5'
73	2,3',5',6
74	2,4,4',5
75	2,4,4',6
76	2,3',4',5'
77	3,3',4,4'
78	3,3',4,5
79	3,3',4,5'
80	3,3',5,5'
81	3,4,4',5

pentaBDE	
82	2,2',3,3',4
83	2,2',3,3',5
84	2,2',3,3',6
85	2,2',3,4,4'
86	2,2',3,4,5
87	2,2',3,4,5'
88	2,2',3,4,6
89	2,2',3,4,6'
90	2,2',3,4',5
91	2,2',3,4',6
92	2,2',3,5,5'
93	2,2',3,5,6
94	2,2',3,5,6'
95	2,2',3,5',6
96	2,2',3,6,6'
97	2,2',3,4',5'
98	2,2',3,4',6'
99	**2,2',4,4',5**
100	**2,2',4,4',6**
101	2,2',4,5,5'
102	2,2',4,5,6'
103	2,2',4,5',6
104	2,2',4,6,6'
105	2,3,3',4,4'
106	2,3,3',4,5
107	2,3,3',4',5
108	2,3,3',4,5'
109	2,3,3',4,6
110	2,3,3',4',6
111	2,3,3',5,5'
112	2,3,3',5,6
113	2,3,3',5',6
114	2,3,4,4',5
115	2,3,4,4',6
116	2,3,4,5,6
117	2,3,4',5,6
118	2,3',4,4',5
119	2,3',4,4',6
120	2,3',4,5,5'
121	2,3',4,5',6
122	2,3,3',4',5'
123	2,3',4,4',5'
124	2,3',4',5,5'
125	2,3',4',5',6
126	3,3',4,4',5
127	3,3',4,5,5'

hexaBDE	
128	2,2',3,3',4,4'
129	2,2',3,3',4,5
130	2,2',3,3',4,5'
131	2,2',3,3',4,6
132	2,2',3,3',4,6'
133	2,2',3,3',5,5'
134	2,2',3,3',5,6
135	2,2',3,3',5,6'
136	2,2',3,3',6,6'
137	2,2',3,4,4',5
138	2,2',3,4,4',5'
139	2,2',3,4,4',6
140	2,2',3,4,4',6'
141	2,2',3,4,5,5'
142	2,2',3,4,5,6
143	2,2',3,4,5,6'
144	2,2',3,4,5',6
145	2,2',3,4,6,6'
146	2,2',3,4',5,5'
147	2,2',3,4',5,6
148	2,2',3,4',5,6'
149	2,2',3,4',5',6
150	2,2',3,4',6,6'
151	2,2',3,5,5',6
152	2,2',3,5,6,6'
153	**2,2',4,4',5,5'**
154	**2,2',4,4',5,6'**
155	2,2',4,4',6,6'
156	2,3,3',4,4',5
157	2,3,3',4,4',5'
158	2,3,3',4,4',6
159	2,3,3',4,5,5'
160	2,3,3',4,5,6
161	2,3,3',4,5',6
162	2,3,3',4',5,5'
163	2,3,3',4',5,6
164	2,3,3',4',5',6
165	2,3,3',5,5',6
166	2,3,4,4',5,6
167	2,3',4,4',5,5'
168	2,3',4,4',5',6
169	3,3',4,4',5,5'

heptaBDE	
170	2,2',3,3',4,4',5
171	2,2',3,3',4,4',6
172	2,2',3,3',4,5,5'
173	2,2',3,3',4,5,6
174	2,2',3,3',4,5,6'
175	2,2',3,3',4,5',6
176	2,2',3,3',4,6,6'
177	2,2',3,3',4,5',6'
178	2,2',3,3',5,5',6
179	2,2',3,3',5,6,6'
180	2,2',3,4,4',5,5'
181	2,2',3,4,4',5,6
182	2,2',3,4,4',5,6'
183	**2,2',3,4,4',5',6**
184	2,2',3,4,4',6,6'
185	2,2',3,4,5,5',6
186	2,2',3,4,5,6,6'
187	2,2',3,4',5,5',6
188	2,2',3,4',5,6,6'
189	2,3,3',4,4',5,5'
190	2,3,3',4,4',5,6
191	2,3,3',4,4',5',6
192	2,3,3',4,5,5',6
193	2,3,3',4',5,5',6

octaBDE	
194	2,2',3,3',4,4',5,5'
195	2,2',3,3',4,4',5,6
196	2,2',3,3',4,4',5,6'
197	2,2',3,3',4,4',6,6'
198	2,2',3,3',4,5,5',6
199	2,2',3,3',4,5,5',6'
200	2,2',3,3',4,5,6,6'
201	2,2',3,3',4,5',6,6'
202	2,2',3,3',5,5',6,6'
203	2,2',3,4,4',5,5',6
204	2,2',3,4,4',5,6,6'
205	2,3,3',4,4',5,5',6

nonaBDE	
206	2,2',3,3',4,4',5,5',6
207	2,2',3,3',4,4',5,6,6'
208	2,2',3,3',4,5,5',6,6'

decaBDE	
209	**2,2',3,3',4,4',5,5',6,6'**