SCIENTIFIC OPINION

Scientific Opinion on Hexabromocyclododecanes (HBCDDs) in Food[1]

EFSA Panel on Contaminants in the Food Chain (CONTAM)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific output, published on 28 July 2011, replaces the earlier version published on 26 July 2011.[4]

ABSTRACT

EFSA was asked by the European Commission to deliver a scientific opinion on hexabromocyclododecanes (HBCDDs) in food. HBCDDs are additive flame retardants primarily used in expanded and extruded polystyrene applied as construction and packing materials, and in textiles. Technical HBCDD predominantly consists of three stereoisomers (α-, β- and γ-HBCDD). Also δ- and ε-HBCDD may be present but at very low concentrations. HBCDDs are present in the environment and likewise in biota and in food and feed. Data from the analysis of HBCDDs in 1,914 food samples were provided to EFSA by seven European countries, covering the period from 2000 to 2010. The Panel on Contaminants in the Food Chain (CONTAM Panel) selected α-, β- and γ-HBCDD to be of primary interest. Since all toxicity studies were carried out with technical HBCDD, a risk assessment of individual stereoisomers was not possible. Main targets were the liver, thyroid hormone homeostasis and the reproductive, nervous and immune systems. HBCDDs are not genotoxic. The CONTAM Panel identified neurodevelopmental effects on behaviour as the critical endpoint, and derived a benchmark dose lower confidence limit for a benchmark response of 10 % ($BMDL_{10}$) of 0.79 mg/kg body weight. Due to the limitations and uncertainties in the current data base, the CONTAM Panel concluded that it was inappropriate to use this BMDL to establish a health based guidance value, and instead used a margin of exposure (MOE) approach for the health risk assessment of HBCDDs. Since elimination characteristics of HBCDDs in animals and humans differ, the Panel used the body burden as starting point for the MOE approach. The CONTAM Panel concluded that current dietary exposure to HBCDDs in the European Union does not raise a health concern. Also additional exposure, particularly of young children, to HBCDDs from house dust is unlikely to raise a health concern.

[1] On request from the European Commission, Question No EFSA-Q-2010-00218, adopted on 5 July 2011.

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[4] The term “intra-individual” has been replaced by “individual” in the Summary and in Chapter 9.1.

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KEY WORDS

Hexabromocyclododecanes, HBCDDs, occurrence, food, toxicology, human exposure, risk assessment

SUMMARY

Following a request from the European Commission, the Panel on Contaminants in the Food Chain (CONTAM Panel) was asked to deliver a scientific opinion on hexabromocyclododecanes (HBCDDs) in food. HBCDDs are stereoisomers of 1,2,5,6,9,10-hexabromocyclododecane. Technical HBCDD predominantly consists of three stereoisomers (α-, β- and γ-HBCDD) present in relative amounts of 9-13 % α-HBCDD, <0.5-12 % β-HBCDD and 72-90 % γ-HBCDD. Also δ- and ε-HBCDD may be present in the technical product, although at very low concentrations. The HBCDD stereoisomers occur as enantiomer pairs.

HBCDDs constitute an important and widely used group of additive flame retardants primarily used in expanded and extruded polystyrene applied as construction and packing materials, and also used in textiles. Concentrations in products are usually in the range between 0.7 % and 3.0 % by weight. As they are mixed into polymers and not chemically bound to the plastic or textiles, HBCDDs might leach from the products into the environment. Technical HBCDD has been on the market since the 1960s but the use in insulation boards started in the 1980s.

HBCDD stereoisomers are susceptible to elimination of HBr and reductive debromination. Abiotic transformation of γ-HBCDD to α-HBCDD has been shown, and it can be concluded that α-HBCDD is the most persistent of the three main HBCDD stereoisomers.

Based on the composition of technical HBCDD and occurrence of the stereoisomers in food and the environment, the CONTAM Panel selected α-, β- and γ-HBCDD to be of primary interest for this opinion.

Following an advice of the CONTAM Panel, a monitoring programme was carried out starting in 2006 and results obtained from the analysis of HBCDDs in 1,914 food samples were provided by seven European countries, covering the period from 2000 to 2010. 'Fish and other seafood' was the food group with the highest number of samples followed by 'Meat and meat products, 'Milk and dairy products' and 'Eggs and egg products'. There were only few occurrence data in the food group 'Food for infants and small children'. The data were characterised by a high proportion of non-detects. The analytical results were reported as total HBCDD or as α-HBCDD, β-HBCDD and γ-HBCDD, whereas the sum of the three individual stereoisomers was calculated for the purpose of risk assessment. Overall, α-HBCDD is the main contributor to the total levels of HBCDDs in all food categories.

For the food groups of 'Eggs and eggs products', 'Milk and dairy products' and 'Meat and meat products (including edible offal)' the lower bound (LB) and upper bound (UB) of the sum of the three individual stereoisomers are 0.14 and 0.54 ng/g fat, 0.03 and 0.67 ng/g fat, 0.14 and 0.79 ng/g fat, respectively. For these food categories the sum of the three stereoisomers is higher than the reported values of total HBCDD. The CONTAM Panel noted that the high proportion of non-detects has an impact on the sum of the three stereoisomers, particularly on the UB estimates.

For the food group of 'Fish and other seafood' the CONTAM Panel concluded that the estimated mean LB and UB of the reported total HBCDD were a more realistic estimation of levels of HBCDDs in fish than the sum of individual stereoisomers. The LB and UB of the reported mean for total HBCDD are 0.98 and 1.16 ng/g wet weight.

The mean dietary exposure to HBCDDs across dietary surveys in European countries was estimated for children from three to ten years old ('Other children') ranging from 0.15 to 1.85 ng/kg body weight (b.w.) per day for the minimum LB and maximum UB, respectively. Total dietary exposure for adults is about half the exposure for 'Other children, with minimum LB and maximum UB of,

respectively, 0.09 and 0.99 ng/kg b.w. per day. Dietary exposure to HBCDDs is decreasing with increasing age down to 0.06 and 0.54 ng/kg b.w. per day for the minimum LB and maximum UB, respectively, for 'Very elderly' (from 75 years of age and older).

Similar exposure patterns across age classes are found for the dietary intake of high consumers (95th percentile). The minimum LB and maximum UB dietary intake of HBCDDs across dietary surveys in European countries are, respectively, 0.80 and 4.46 ng/kg b.w. per day for 'Other children', followed by 'Adults' with 0.39 and 2.07 ng/kg b.w. per day, and down to 0.27 and 1.26 ng/kg b.w. per day for the 'Very elderly'.

For a specific population group consisting of high consumers of fish the total mean dietary UB intake of HBCDDs (maximum UB across European surveys) is 2.76 ng/kg b.w. per day. The total dietary UB intake of consumers of fish liver (once a week) is estimated to be 1.94 ng/kg b.w.

As contamination of food samples of plant origin is generally lower than that of food samples of animal origin, it can be assumed that the dietary exposure of vegetarians to HBCDDs is lower than that for people consuming a mixed diet.

For breast-fed infants with average human milk consumption (800 mL per day) the reported range for total HBCDD in human milk (0.13-31 ng/g fat) results in daily exposures of 0.60-142 ng/kg b.w. For infants with high human milk consumption (1,200 mL per day) this is 0.90-213 ng/kg b.w.

The available toxicokinetics data suggest that orally administered HBCDD is easily absorbed and rapidly distributed in different tissues, with some differences observed between γ- and α-stereoisomer. In contrast to γ-HBCDD, α-HBCDD was found to concentrate in adipose tissue. Debromination and hydroxylation seem to be the major metabolic pathways for HBCDDs, but stereoisomerisation of γ-isomer to α- and β-isomers was observed in mice treated with γ-HBCDD. No stereoisomerisation of α-HBCDD was reported.

Calculation of elimination half-lives of HBCDD stereoisomers in female mice, based on concentrations in adipose tissue, vary from 3-4 days for γ-HBCDD, to 17 days for α-HBCDD. The half-life in humans for HBCDDs (reported as sum of α-, β- and γ-HBCDD) was estimated to be 64 days (range 23-219 days). This difference in kinetics affects the extrapolation of animal data to humans.

Toxicological studies have been carried out using different experimental designs with single or repeated administration during gestation, postnatally or in adulthood using technical HBCDD. The composition of these mixtures differs from the HBCDD profile found in wildlife and in foods. Main targets for HBCDD toxicity were the liver, thyroid hormone homeostasis, the reproductive, the nervous and the immune systems.

The activation of constitutive androstane receptor- (CAR) or pregnane-X-receptor (PXR)-dependent gene expression, leading to disruption of thyroid hormone homeostasis, is considered to be associated with neurodevelopmental effects on behaviour and may also be responsible for effects on reproduction.

The available studies indicate that HBCDDs are not genotoxic.

There is limited information from only one long-term toxicity/carcinogenicity study for HBCDDs in B6C3F1 mice, indicating that the incidence of altered foci in the liver of males was increased, as was the incidence of liver carcinoma in females, but without a dose-relationship. The CONTAM Panel noted that the incidence of liver carcinoma was within the range of background levels for this strain of mice. Given the lack of genotoxicity the Panel concluded that carcinogenicity is not a critical effect in the hazard characterisation of HBCDDs.

The two available epidemiological studies did not show any association between the levels of HBCDDs in blood and bone mineral density in an elderly female population, and between levels of HBCDDs in human milk and effects on neonatal thyroid-stimulating hormone (TSH).

Since all toxicity studies were carried out with technical HBCDD, a risk assessment of individual stereoisomers was not possible.

Based on the information from animal experiments the CONTAM Panel identified neurodevelopmental effects on behaviour in mice, observed in a study with single administration of technical HBCDD on postnatal day (PND) 10, as the critical end-point and derived a benchmark dose lower confidence limit for a benchmark response of 10 % ($BMDL_{10}$) of 0.93 mg/kg b.w. to be used as reference point for the hazard characterisation.

Because elimination kinetics of HBCDDs in rodents and humans differ, external dose levels of HBCDDs associated with toxic effects in animals cannot be simply extrapolated for the risk assessment in humans. Instead, the internal dose or body burden provides a more appropriate dose metric for a direct comparison of effects in animals and humans. Based on the calculated $BMDL_{10}$ value of 0.93 mg/kg b.w. as derived from a study using a single oral administration, and considering an oral absorption of in rodents of 85 %, a body burden at the $BMDL_{10}$ of 0.79 mg/kg b.w. was derived.

This body burden estimate could in principle be used as the basis to establish a health based guidance value, e.g. a tolerable daily intake. The CONTAM Panel concluded however, that due to the limitations and uncertainties in the current data base on HBCDDs, the derivation of a health based guidance value was not appropriate. Instead, the Panel used a margin of exposure (MOE) approach for the risk characterisation of HBCDDs, by comparing the minimum LB and maximum UB dietary intake for HBCDDs with the estimated human intake associated with the body burden at the $BMDL_{10}$.

The maximum UB dietary intake for average and high adult consumers results in an MOE of about 3,000 and 1,450, respectively. For high fish consumers the MOE is 1,000 and for regular consumers of fish liver 1,500. For children of the age of 3-10 years with an average and high consumption, the maximum UB dietary intake results in an MOE of 1,600 and 700, respectively.

Usually a MOE of 100, covering uncertainties and variability with respect to kinetic and dynamic differences between animal species and humans (factor $4 \times 2.5 = 10$) and within the human population (factor $3.2 \times 3.2 = 10$), is considered sufficient to conclude that there is no health concern. Since the MOE approach is based on a body burden comparison between animals and humans, the potential kinetic differences have been accounted for. Equally, by focusing on the body burden associated with a $BMDL_{10}$ for neurobehavioural effects in mice induced during a relevant period for brain development, and applying this body burden to the entire life span in humans, individual difference in susceptibility has been covered. Therefore, the calculated MOE should be sufficient to cover inter-species differences in dynamics for the effects observed (factor 2.5). Considering the uncertainty in the elimination half-life in humans the CONTAM Panel concluded that the MOE should also cover individual differences in kinetics (factor 3.2). This implies that an MOE larger than 8 (2.5×3.2) might indicate that there is no health concern.

The calculated MOEs for the various sub-populations, based on the maximum UB dietary intake, are in the range of 700 to 3,000 and thus much larger than a factor of 8. In addition, the CONTAM Panel noted that use of the UB estimates would have resulted in an overestimation of the risk. Therefore it was concluded that current dietary exposure to HBCDD does not raise a health concern.

For breast-fed infants with average or high human milk consumption MOEs ranging from 21 to 5,000 and from 14 to 3,300 have been estimated. The lowest values for these MOEs are about 2.6 and 1.7 fold larger than a factor of 8. Also considering that consumption of human milk occurs only

through a restricted period in life, the CONTAM Panel concluded that it is unlikely that exposure via human milk raises a health concern.

The CONTAM Panel was not able to assess the dietary intake of infants (< 1 year) and toddlers (1-3 years) but assumed that dietary intake of contaminants such as HBCDDs of these age groups usually is lower than that of breast-fed infants. Therefore it concluded that it is also unlikely that dietary exposure to HBCDDs of infants and toddlers will raise a health concern.

Due to the uncertainty regarding the estimated half life of HBCDDs in humans, the CONTAM Panel also considered information on biomarkers of exposure for comparison with the outcome of the MOE approach. It identified information on total HBCDD concentrations in adipose tissue as being most relevant, because they best reflect long-term exposure to HBCDDs. Concentrations in adipose tissue range from <0.5 to 7.5 ng/g fat. When these values are converted into body burden concentrations assuming a fat content of 25 % for the human female adult body, and these body burdens are compared with the body burden at the $BMDL_{10}$ of 0.79 mg/kg b.w., a margin of 420 to >6,300 can be estimated. A similar range is found using data of HBCDDs in human serum. The CONTAM Panel concluded that this result supports the conclusion that current dietary exposure to HBCDDs in the EU does not raise a health concern.

Dust in homes, classrooms and cars can be an additional source of exposure to HBCDDs for children. Based on a 'typical' exposure scenario, ingestion of HBCDDs by young children (1-6 years old) via dust has been estimated to be about 5.9 ng/kg b.w. per day. In the same study a 'high' exposure scenario resulted in an estimated daily exposure through dust of 330 ng HBCDDs/kg b.w. The CONTAM Panel noted however that considerably lower intake estimates for HBCDDs from dust has been reported in another study. It therefore concluded that the 'typical' exposure scenario provided the most realistic estimate of exposure to HBCDDs from dust. This 'typical' exposure scenario results in a MOE of about 500 for exposure to dust only. Combining the UBs of average or high dietary intake of children of this age group with this 'typical' dust exposure leads to a total exposure to HBCDDs of about 7.7 and 10.3 ng/kg b.w., respectively. The resulting MOEs are about 390 and 300, respectively. Taking into account the uncertainties in the dust exposure estimates and considering the use of UB dietary intake estimates, the CONTAM Panel concluded that the available information indicates that it is unlikely that additional exposure to HBCDDs from dust raises a health concern.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Brominated flame retardants (BFRs) are anthropogenic chemicals that are added to a wide variety of consumer/commercial products in order to improve their fire resistance. There are 5 major classes of BFRs: brominated bisphenols, diphenyl ethers, cyclododecanes, phenols and phthalic acid derivatives.

Concern has been raised because of the occurrence of several chemical compounds from the group of BFRs in the environment, including feed and food, and in human biota. This has led to bans on the production and use of certain formulations of polybrominated diphenyl ethers (PBDEs).

EFSA concluded in its advice on a request from the Commission related to relevant chemical compounds in the group of brominated flame retardants for monitoring in feed and food of 24 February 2004 that the available occurrence data on brominated flame retardants in feed and food did not allow a comprehensive assessment of contamination in all feeds and foods and identified the following compounds as the most important ones to be monitored based on the analytical feasibility to measure the chemical compounds routinely in accredited laboratories, the production volumes, the occurrence of the chemical compounds in food and feed, their persistence in the environment and their toxicity:

- polybrominated diphenyl ethers (PBDEs): BDE congeners #28, 47, 99, 100, 153, 154, 183 and 209;
- hexabromocyclododecane (HBCD): total amount (isomer specific analysis of a limited number of samples and/or pools in case of significantly elevated levels or increasing trends);
- polybrominated biphenyls (PBBs): BB congener #153.

Optionally, the following brominated flame retardants were recommended to be monitored:

- TBBP-A and other phenols;
- decabromodiphenyl ethane;
- hexabromobenzene;
- bis(2,4,6-tribromophenoxy)ethane.

Subsequently EU-wide monitoring of these compounds was organised as of October 2006. Monitoring results will be made available to EFSA.

In order to assess the need for regulatory measures as regards BFR in food, EFSA is requested to assess the risks related to the presence of BFR in food.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Art 29 (1) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority for a scientific opinion on the risks to human health related to the presence of hexabromocyclododecane (HBCD) in food.

In particular, the opinion should

- evaluate the toxicity of the HBCD for humans considering all relevant toxicological information available;

- carry out an exposure assessment on the basis of the occurrence data obtained in the monitoring exercise and other occurrence data that may be available;

- consider the exposure situation for specific groups of the population (e.g. infants and children, people following specific diets, etc.) and indicate the relative importance from other non-dietary sources;

- take into account, if available, biomonitoring data when assessing the exposure and compare the results with the calculated exposure;

- explore whether individual compounds can be used as markers for dietary exposure to BFRs;

- identify potential data gaps for these specific groups of BFRs.

ASSESSMENT

1. Introduction

1.1. General information

Flame retardants include a broad and diverse group of compounds used to prevent fires or at least to slow down the development of a fire. There are three main categories of chemical flame retardants: halogenated hydrocarbons, organophosphorous compounds and inorganic products often based on metallic hydroxides (Vos et al., 2003). Within the halogenated hydrocarbons, the group of the brominated flame retardants (BFRs) consist of different chemicals with a variety of physicochemical properties and uses. The main BFRs are the polybrominated (i) neutral aromatic, (ii) neutral cycloaliphatic, (iii) phenolic, including neutral derivatives, (iv) aromatic carboxylic acid esters, and (v) trisalkyl phosphate compounds. The major individual groups of BFRs within these five classes are, respectively, tetrabromobisphenol A (TBBPA), polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDDs[5]), decabromodiphenyl ethane (DBDPE), bis(2,4,6-tribromophenoxy)ethane (BTBPE) and 2,4,6-tribromophenol (WHO, 1997; Örn and Bergman, 2004; Harju et al., 2009). A set of 10-20 other BFRs comprise a group of miscellaneous brominated compounds.

The present opinion will focus on HBCDDs, i.e. HBCDD stereoisomers. Technical HBCDD consists primarily of the γ-stereoisomer with some α- and β-HBCDD. Apart from these three major stereoisomers, also δ- and ε-HBCDD stereoisomers may be present in the technical HBCDD product, although at very low concentrations. Technical HBCDD is used under various commercial names as an additive flame retardant to polymers of different types, primarily polystyrene, and for textile coating (ECB, 2008). The HBCDDs stereoisomers are not chemically bound to the polymers, and they can therefore leach into the environment.

Based on the composition of technical HBCDD, occurrence of the stereoisomers in the environment and available data on their toxicity, the Panel on Contaminants in the Food Chain (CONTAM Panel) selected α-, β- and γ-HBCDDs to be of primary interest for this opinion.

In this opinion the term 'total HBCDD' is used for data that are generated by analytical techniques which cannot separate the various stereoisomers but determine these as a single compound. In contrast, the term 'sum of HBCDDs' is used for results that are generated by stereoisomer-specific analysis and subsequently summed.

1.2. Previous risk assessments

In 2008, the European Chemicals Bureau (ECB) issued a risk assessment report on the risk to the environment and human health from exposure to HBCDDs (ECB, 2008). The report concluded that HBCDDs lacked significant genotoxic potential *in vitro* as well as *in vivo*. No adequately performed cancer study was identified. The developmental toxicity studies evaluated, did not demonstrate any fetotoxicity, teratogenic potential or adverse effects on development of rats, although increased pup mortality during lactation was observed in one study. Possible developmental neurotoxic effects (statistically significant changes in spontaneous behaviour, learning and memory defects) were reported after neonatal exposure to HBCDDs with a lowest-observed-adverse-effect level (LOAEL) of

[5] HBCDDs is used as the abbreviation for hexabromocyclododecanes (1,2,5,6,9,10-hexabromocyclododecane, CAS No 3194-55-6) instead of HBCD in this document, to avoid misunderstandings. HBCD is occasionally used as an abbreviation of hexabromocyclodecane (CAS No 25495-98-1).

0.9 mg/kg body weight (b.w.) per day (Eriksson et al., 2006). However, the Report concluded that since the study, although well performed, did not follow the current guidelines the results needed to be confirmed by other laboratories. The end-points of concern identified for risk characterisation were (i) increased liver weight for which a benchmark dose lower confidence limit for a benchmark response of 20 % ($BMDL_{20}$) of 22.9 mg/kg b.w. per day was deduced from a 28-day oral study using a benchmark model design (van der Ven et al., 2006) and (ii) reproductive toxicity/fertility for which a no-observed-adverse-effect level (NOAEL) of 10 mg/kg b.w. per day was deduced from a two-generation reproductive toxicity study in rats (Ema et al., 2008).

The intake of HBCDDs via food was estimated to be 0.02 µg/kg b.w. per day, based on measured values in food basket studies. Root-crops and fish were the foodstuffs contributing most to the intake. When comparing this intake to the established NOAELs for repeated dose toxicity (increase liver weight) and reproductive toxicity/fertility, margins of safety (MOSs) of 1.15×10^6 and 0.5×10^6 were obtained, respectively. For breast-fed infants, the worst case average daily intake was estimated to be 0.015 µg/kg b.w. per day, based on Swedish human milk data. This exposure level was compared to a NOAEL of 22.9 mg/kg b.w. per day for repeated dose toxicity, giving a MOS of 1.5×10^6, or to a NOAEL of 10 mg/kg b.w. per day for reproductive toxicity/fertility, giving a MOS of 7×10^5. It was concluded that these large MOSs indicate that there is no concern for repeated dose toxicity and reproductive toxicity for breast-fed infants.

It was also noted that although α-HBCDD is the major isomer in the exposure of humans via the environment, it is a minor component of the technical HBCDD used in most of the toxicological studies.

In 2010, Environment Canada assessed the risk over a lifetime from exposure to HBCDDs of the general population of Canada (Environment Canada, 2010). The human health assessment was based on the assessment from the ECB, with more recent data taken into consideration. The critical effect for the risk characterisation was reproductive toxicity, including decreased fertility and thyroid effects. A NOAEL of 10 mg/kg b.w. per day was derived from the study from Ema et al. (2008). The comparison of this critical effect level and the upper bound estimate of the exposure (0.047 µg/kg b.w. per day) resulted in a margin of exposure (MOE) of 213,000. For infants and children it was considered appropriate to use a LOAEL of 0.9 mg/kg b.w. derived from the study from Eriksson et al. (2006). The upper bound exposure estimate for breast-fed infants was 0.11 µg/kg b.w. per day giving a MOE of 8,200. These margins of exposure were considered adequately protective for human health considering the uncertainties in the exposure and health effects databases.

1.3. Chemical characteristics

HBCDDs are stereoisomers substituted with six bromine atoms in the cyclododecane molecule (Figure 1). HBCDD stereoisomers occur as enantiomer pairs (Becher, 2005; Heeb et al., 2007). HBCDDs are formed via addition of bromine to 1,5,9-cyclododecatriene. Technical products of HBCDDs (containing 73-74 % Br) predominantly consists of three stereoisomers (α-, β- and γ-HBCDD) present in relative amounts of 9-13 % α-HBCDD, <0.5-12 % β-HBCDD and 72-90 % γ-HBCDD (Peled et al., 1995). Apart from these three, also δ- and ε-HBCDD may be present in the technical product, although at very low concentrations (Arsenault et al., 2007; Law et al., 2005). In addition, the commercial HBCDDs may contain 1-2 % tetrabromocyclododecene (Peled et al., 1995).

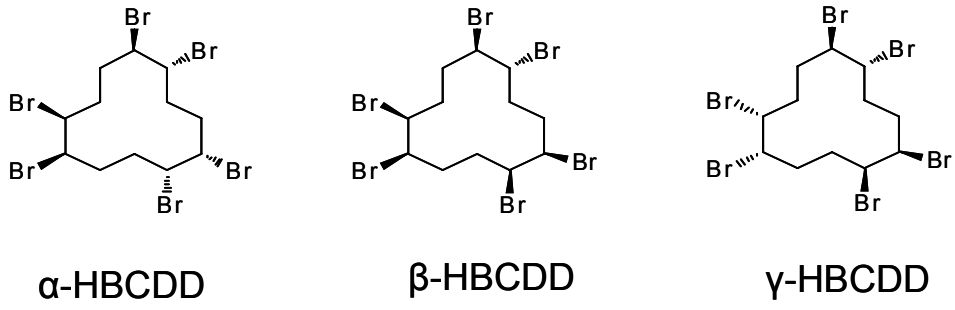

Figure 1: General structure of the three major HBCDD stereoisomers; α-HBCDD, β-HBCDD and γ-HBCDD.

The majority of scientific references to HBCDDs are linked to hexabromocyclododecane (CAS: 25637-99-4) and 1,2,5,6,9,10-hexabromocyclododecane (CAS: 3194-55-6). The development of the HBCDD research has included stepwise improvements of identifications of stereoisomers, which all together has lead to a list of at least 15 CAS numbers of HBCDDs, not including CAS numbers related to HBCDDs labelled with stable and radioactive isotopes (Appendix A).

The molecular structures of the six enantiomers, identified as α-, β- and γ-HBCDD (Koeppen et al., 2007; Heeb et al., 2007) are shown in Figure 2.

The HBCDD stereoisomers have a molecular mass of 641.7 g/mol, an octanol-water partitioning coefficient (Log K_{ow}) of 6.6 and a vapour pressure of 7.8×10^{-10} Torr at 25°C, the latter two according to model calculations (Advanced Chemistry development (ACD/Labs) Software V11.02 (© 1994-2011 ACD/Labs). Log K_{ow} as reported by ECB (2008) are 5.07, 5.12 and 5.47 for α-, β- and γ-HBCDD, respectively, while modelled by COSMOtherm to be 5.59, 5.44 and 5.53, respectively (Goss et al., 2008). HBCDD stereoisomers are non-ionisable compounds that do not absorb ultraviolet (UV) light above 290 nm. The water solubility of the HBCDD stereoisomers is 48.8 ± 1.9 µg/L for α-HBCDD, 14.7 ± 0.5 µg/L for β-HBCDD and 2.1 ± 0.2 µg/L for γ-HBCDD. For the HBCDD technical product the solubility is 66 µg/L (MacGregor and Nixon, 2004, as cited by ECB, 2008). The full set of physicochemical characteristics is given in the ECB report (ECB, 2008).

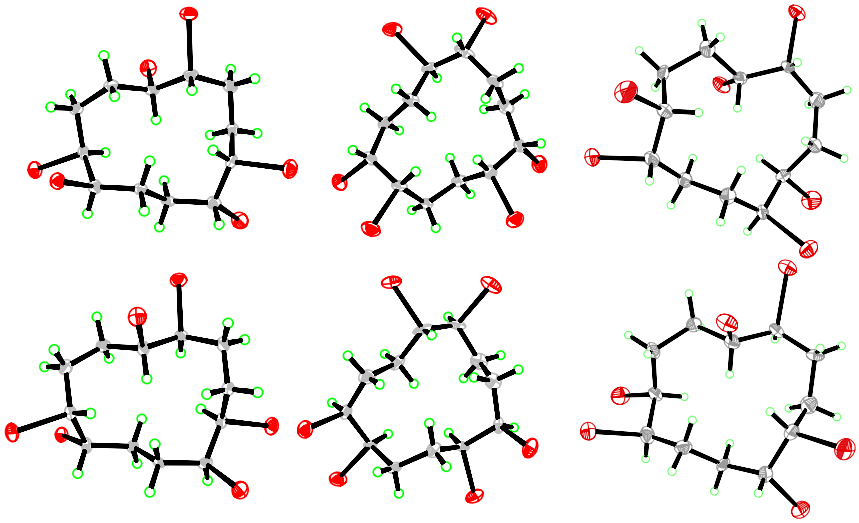

Figure 2: Molecular structure of the HBCDD enantiomers, upper row, left to right: (+)-α-HBCDD, (+)-β-HBCDD, (+)-γ-HBCDD, and lower row, left to right: (-)-α-HBCDD, (-)-β-HBCDD, (-)-γ-HBCDD. Figure prepared for the present document by Roland Becker based on structures reported by Koeppen et al. (2007).

The chemical reactivity of HBCDDs is due primarily to elimination of hydrogen bromide leading to unsaturations (double bonds) in the resulting molecule. Experimental studies have shown that γ-HBCDD is rearranged highly efficiently to α-HBCDD at temperatures of 190ºC (Peled et al., 1995). Trans-1,2-dibromo-substituted compounds are known to undergo thermal rearrangements via a four centre transition state (Peled et al., 1995). β-HBCDD is not affected by the thermal rearrangement reaction (Fång, 2007). Accordingly, this γ-α transformation confirms the high stability of α-HBCDD. Based on the physicochemical and reactivity properties, it can be concluded that α-HBCDD is the most persistent of the three main HBCDD stereoisomers.

2. Legislation

In order to protect public health, Article 2 of Council Regulation (EEC) No 315/93[6] of 8 February 1993 laying down Community procedures for contaminants in food stipulates that, where necessary, maximum tolerances for specific contaminants shall be established. Thus, a number of maximum tolerances are currently laid down in Commission Regulation (EC) No. 1881/2006[7] of 19 December 2006 setting maximum levels (MLs) for certain contaminants in foodstuffs, e.g. dioxins, dioxin-like PCBs and benzo[a]pyrene. HBCDDs are neither regulated so far under this Regulation nor under another EU regulation for food.

Council Directive 2002/32/EC[8] regulates undesirable substances in animal feed. While maximum contents are set for a number of inorganic and organic contaminants in various feed materials, HBCDDs are not regulated so far by the European Commission (EC) under this Directive.

On February 17, 2011 the European Commission decided to list the first group of substances, including HBCDDs, in annex XIV to REACH (Commission Regulation (EC) No 1907/2006[9] concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals. Annex XIV lists substances subject to authorisation. The listing implies that within 3-5 years, specific authorisation will be required for a manufacturer, importer or downstream user to place the substance on the market or to use it in preparations or to incorporate it into articles.

The Persistent Organic Pollutants Review Committee (POPRC) of the Stockholm Convention has concluded that HBCDDs meet the criteria set out in Annex D to the Convention and to adopt the Risk Profile for HBCDDs (UNEP, 2010). The committee also concluded that it is likely that HBCDDs, as a result of its long range environmental transport, can lead to significant adverse human health and environmental effects such that global action is warranted. It was decided to establish an ad hoc working group to prepare a Risk Management Evaluation that includes an analysis of possible control measures for HBCDDs. Decision on the inclusion of HBCDDs into the protocol is expected to be taken during 2011. A similar status and process also applies for the long-range transboundary air pollution on persistent organic pollutants (LRTAP-POPs) protocol.

[6] Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food. OJ L 37, 13.2.1993, p. 1.

[7] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364/5, 20.12.2006, p. 5-24.

[8] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140/10, 30.5.2002, p. 10-21.

[9] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. OJ L 136/3, 29.5.2007.

3. Sampling and methods of analysis

3.1. Sampling

There are no specific guidelines for the sampling of foods to be analysed for their HBCDD content. Therefore, basic rules for sampling of organic contaminants or pesticides should be followed. Respective requirements are for example laid down in Commission Regulation (EC) No 1883/2006[10] of 19 December 2006 laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs. This Regulation contains inter alia a number of provisions concerning methods of sampling depending on the size of the lot, packaging, transport, storage, sealing and labelling. The primary objective is to obtain a representative and homogeneous laboratory sample with no secondary contamination.

3.2. Methods of analysis

Current analytical methods allow the chromatographic separation and determination of all HBCDD stereoisomers (α- to ε-HBCDD). These methods are based on reversed phase liquid chromatography (LC). LC based separation methods of chiral compounds allow analysis of HBCDD enantiomers. HBCDDs can also be determined by gas chromatography (GC), but further separation of stereoisomers is not possible by this approach. Depending on the method used, the concentration is either reported as total HBCDD (GC) or as results for the individual α-, β- and γ-HBCDD stereoisomers, and the sum thereof (LC).

The analytical method starts with the extraction of the HBCDDs from the sample. Several methods for extraction of biological samples have been proposed in the literature as reviewed by Covaci et al. (2007). For extraction of solid material, the Soxhlet procedure is used in some laboratories because it is simple and provides high extraction efficiency. Other techniques include pressurised liquid extraction (PLE), shaking with organic solvent (Nakagawa et al., 2010) and solid-phase extraction (SPE).

Cleanup of the extract is performed to isolate the HBCDDs from the co-extracted interfering compounds such as lipids and other matrix constituents. Co-extracted lipids can be removed by e.g. gel permeation chromatography (GPC), alumina-oxide chromatography and (multilayer) silica chromatography (Covaci et al., 2007). The next step is fractionation to isolate the HBCDDs from other pollutants and potentially interfering compounds. This is typically done by silica column fractionation (Covaci et al., 2007). β-HBCDD requires more solvent than α- and γ-HBCDD for a complete elution from a silica column (Morris et al., 2006; Mariussen et al., 2010). Silica treated with alcoholic NaOH or KOH may cause losses of HBCDDs due to the loss of hydrogen bromide (Covaci et al., 2007; de Boer et al., 2001), leading to one or more unsaturations in the HBCDD stereoisomers.

HBCDDs can be analysed by GC-MS methods. The injection of HBCDDs into the GC system is a critical part of the chromatographic analysis. Splitless injection is the most commonly used technique for GC analysis of HBCDDs. However, the programmable temperature vaporisation injector (PTV) and on-column injectors have also been used successfully in HBCDD GC analysis. At temperatures >190˚C, the ratio between the HBCDD stereoisomers may change (Peled et al., 1995) as discussed in an analytical chemical perspective (Covaci et al., 2003; de Boer and Wells, 2006) and at >240˚C HBCDDs decompose in many different compounds (Morris et al., 2006; Barontini et al., 2001). Therefore, long sample residence times at high temperatures in the injector should be avoided.

GC separations are done on capillary columns with an apolar or slightly polar stationary phase. The column dimensions are typically 25-60 m length, 0.25 mm diameter and 0.1-0.25 µm film thickness. HBCDDs may degrade in the GC column to pentabromocyclododecene and

[10] Commission Regulation (EC) No 1883/2006 of 19 December 2006 laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs. OJ L 364/32, 20.12.2006, p. 32-43.

tetrabromocyclododecadiene. This is demonstrated for a standard solution of α-, β- and γ-HBCDD stereoisomers by Abdallah et al. (2008) and van Leeuwen (2009). In particular, the degradation of HBCDDs is increased by e.g. high temperatures, time spent at elevated temperatures and presence of catalytic sites when GC analysis is applied. For the best yield of HBCDDs, these parameters should be controlled.

In GC-low resolution MS (LRMS), electron chemical negative ionisation (ECNI) is commonly used as ionisation method for BFRs. This ionisation method provides significantly better sensitivity compared to electron impact (EI) ionization, although at the cost of selectivity (Covaci et al., 2006, 2007). With ECNI-MS, only the bromine isotopes (*m*/*z* 79 and 81) are monitored which hampers the identification of different compounds based on their molecular masses. With EI the $[M\text{-}Br]^-$ ion can be monitored resulting in higher selectivity (Roosens et al., 2008). The latter provides the possibility to use mass labelled standards, but the sensitivity is too low for many environmental samples in the case of LRMS. The analysis of total-HBCDD can also be performed by integrating it into the method for the determination of PBDEs applying GC-high resolution MS (GC-HRMS) (Shaw et al., 2008). MS responses differ for α-, β- and γ-HBCDD stereoisomers, with α-HBCDD showing the highest response. Because of this difference, it is essential that the stereoisomer profile in the calibration solution is equal to the profile in the analysed sample.

For the analysis of the HBCDD stereoisomers, generally reversed phase columns are being used and for the analysis of the enantiomers, enantioselective columns (permethylated-cyclodextrin stationary phases) are required (Janak et al., 2005; Marvin et al., 2007). Enantioselective analysis allows determination of enantiomers and can detect whether enrichment of (+) or (-) enantiomers occurs. For example, Eljarrat et al. (2009) found (-)-α-HBCDD to dominate in human milk samples.

LC-MS detection is mostly performed on triple-quadrupole instruments (MS/MS) using the electrospray ionisation (ESI) source. ESI-ion trap MS instruments (ITMS) have been used as well as atmospheric pressure chemical ionisation (APCI). For an extensive overview of methods used see Covaci et al. (2007). ESI was preferred over APCI by Budakowski and Tomy (2003). In the ESI source, the formation of $[M\text{-}H]^-$ takes place. The MS spectrum exhibits bromine clusters because of the two bromine isotopes *m*/*z* 79 and *m*/*z* 81 present. The most intense peak in the cluster is *m*/*z* 640.7 ($^{12}C_{12}H_{17}{}^{79}Br_3{}^{81}Br_3$). Triple quadrupole MS/MS instruments allow for selective detection by isolation of the precursor ion $[M\text{-}H]^-$ (*m*/*z* 640.6) in the first quadrupole, followed by detection of the bromine isotope $[Br]^-$ (m/*z* 79 and/or 81) in the third quadrupole.

A major advantage of LC-ESI-MS(/MS) and GC-EI-HRMS over GC-ECNI-MS is the option of using ^{13}C labelled internal standards. These standards allow correction for losses during extraction and clean-up. Furthermore, several studies showed that these labelled standards effectively correct for matrix suppression or enhancement occurring in the ESI source (Tomy et al., 2005; Gómara et al., 2007; Marvin et al., 2007).

The results obtained by both techniques for fish samples showed large discrepancies in a study by van Leeuwen and de Boer (2008). GC results were on average 4.4-fold higher than LC results. Discrepancies between GC and several LC methods were also found by Roosens et al. (2008) in eel samples. On the other hand, Päpke et al. (2010) reported on the analysis of food samples by GC-ECNI-MS and LC-MS/MS and found a close agreement between both techniques.

Quality control (QC) and quality assurance (QA)

The analysis of HBCDDs is laborious and complex and involves several critical steps. Errors are easily made in extraction, cleanup, GC determination and quantification. A number of factors determine the final accuracy and precision (i.e. the quality) of the results reported. Exposure to high temperatures should be avoided, as discussed earlier. Exposure to UV radiation may lead to the transformation of γ-HBCDD to α-HBCDD, as was recently reported in dust by Harrad et al. (2009a). Due to this fact, it is recommended that all analytical work is carried out in such a manner that UV

light is kept out, e.g. treatments can be undertaken in brown glass or in glassware covered with aluminium foil. Problems with blanks can be minimised by minimising sample contact with HBCDD treated materials (e.g. polystyrene materials) and to avoid contact with dust particles at every stage of sampling, pre-treatment and analysis. Blank problems are less pronounced than for other BFRs (e.g. BDE-47 and -99).

Interlaboratory studies and certified reference materials (CRMs)

A number of interlaboratory studies have been organised for biota samples by QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe). In many cases, HBCDD levels in the samples were too low to allow an assessment of intercomparability (QUASIMEME 2009, 2010).

The Norwegian Institute of Public Health has organised interlaboratory comparison studies on persistent organic pollutants (POPs) in food, including HBCDDs in food commodities and standard solutions. In the interlaboratory comparison studies organised in 2005 and 2007, the test materials provided to the laboratories were (in the order of decreasing concentrations) a cod liver oil, Baltic Sea herring filet, salmon fillet, butter and chicken meat (Haug et al., 2008). The mean and median GC-MS results of cod liver oil and herring fillet were 10-40 % higher as compared to LC-MS results. In salmon, the mean results were similar. However, it should be noted different laboratories used different techniques (i.e. no laboratory used both techniques), which makes comparison difficult. The variation (relative standard deviation, RSD) of the overall dataset was good for total-HBCDD and sum-HBCDD (20-32 %) and α-HBCDD (24 %) in cod liver oil and herring, but worse for γ-HBCDD (85-88 %). Values for salmon and chicken were somewhat worse. These higher RSD values were explained by the lower levels in the samples and the limited use of mass labelled internal standards. The interlaboratory comparison in 2010 covered pork meat, trout filet, human milk and standard solution with known concentrations of α-HBCDD (Horpestad Liane and Becher, 2010). Thirteen laboratories reported data on HBCDD concentrations (individual stereoisomers and total HBCDD) in the standard solution, while a variable number of laboratories (between 8 and 14) reported data in the various matrices. Since only few laboratories reported concentration of HBCDDs, the values were regarded as indicative.

Standard or Certified Reference Materials (SRM, CRM) are important tools for laboratory performance evaluation against external references. One SRM certified for α- and β-HBCDD freeze dried mussel tissue (SRM 2974a) is available from the National Institute of Standards and Technology. No other food-type CRMs are available for HBCDDs.

4. Sources, use and environmental fate

HBCDD is an important and widely used flame retardant, primarily for application in polystyrene where it is used as an additive at concentrations between 0.7 % and 3.0 % by weight. HBCDDs are mixed with the polymers, and may thus leach from the product applications into the environment. Technical HBCDD has been on the market since the 1960s but the use in insulation boards did not start until the 1980s (ECHA, 2009a). It has not bee possible to identify further detailed historic information available on HBCDD production.

4.1. Formation and production

There are no natural sources of any HBCDDs. According to the producers, HBCDDs are manufactured by bromination of the starting material *cis,trans,trans*-1,5,9-cyclododecatriene (ECB, 2008). The reaction occurs at 20-70˚C in closed systems as a batch process and results in a mixture of compounds that has to be extensively washed and centrifuged before the liquid can be removed for reprocessing and HBCDD can be isolated and dried.

Technical HBCDD is a white odourless solid compound which is used on its own, or in combination with other flame retardants. The ratios between α-HBCDD, β-HBCDD and γ-HBCDD are dependent on the chemicals manufacturing conditions in processing plants. HBCDD have and is sold under many trade names. Some of these names are listed in Table 1.

Table 1: Some common trade names under which technical HBCDD has been or is still marketed. Table is based on data in ECB (2008) and SciFinder CAS and CAplus as given by Säll (2010).

BRE 5300	HBCD IHM	Pyroguard SR 103
Bromkal 73-6CD	HBCD ILM	Pyroguard SR 103A
Bromkal 73-6D	HBCD-LM	Pyroguard SR 103HR
Great Lakes CD 75	HBCD-LMS	Pyroguard SR 104
Great Lakes CD 75P	HBCD-SP 75	Pyrovatex 3887
Great Lakes D75XF	Hexabromid S	SP 75
Great Lakes D75PC	HP 900	SR 103
FR 1010	HP 900G	Safron 5261
FR 104	Hexabromocyclododecane	Saytex HBCD
FR 1206	Myflam 11645	Saytex HBCD-LM
FR 1206HT	Nicca Fi-None CG 1	Saytex HBCD-SF
FR 1206 I-CM	Nicca Fi-None TS 1	Saytex HP 900
FR 1206ILM	Nicca Fi-None TS 3	Saytex HP 900G
FR 1206I-LM	Nicca Fi-None TS 88	YM 88
FR-CD	Pyroguard F 800	YM 88A

According to the risk assessment report of the ECB (2008), in 2008 HBCDD was produced in the EU at one factory in the Netherlands only. In 2005 annual production of HBCDD in the EU was assumed to be 6,000 tonnes. Two other European factories had been closed down in 1997 and 2003, respectively. The global production in 2008 was around 13,400 tonnes annually (Steukers, personal communication, 2008).

HBCDDs were imported to and probably also exported from EU, both as a chemical and in articles. The HBCDD Industry User Group has provided data cited by ECHA (2009a) on the total sales and consumption of HBCDD in all EU27 countries. These data are summarised in Table 2.

Table 2: Sales of HBCDDs in the EU based on data from HBCDD Industry Users Group as cited by ECHA (2009a).

Year	Sales in EU Countries, tonnes
2003	9,448
2004	10,123
2004	10,622
2006	10,075
2007	11,186

There was obviously an increase in the sales of technical HBCDD between 2003 and 2007 of almost about 20 %. No information has been identified on the production since 2007 (except from Steukers, personal communication, 2008). As the production of HBCDDs in the Member States between 2003 and 2007 was rather constant around 6,000 tonnes per year, the net import has increased by of almost 50 % over the period. The best estimate of imports of HBCDDs in 2006 is 5,580 tonnes. Based on these figures, the total consumption in the EU since the 1980s could be in the order of 200,000 tonnes.

4.2. Use

Technical HBCDD is used as an additive flame retardant for protection during the service life of vehicles, buildings or articles. The main uses of HBCDDs are in polystyrene foam insulation widely used in the building and construction industry. The polystyrene foam exist in two main forms, as expanded polystyrene (EPS) and extruded polystyrene (XPS) foams. The manufacture of EPS and XPS involves polymerisation and extrusion processes where HBCDDs are added in the process as one of the additives (ECHA, 2009a). The use in textiles and electric and electronic appliances is smaller (ECHA, 2009a; US-EPA, 2008; OECD, 2007; BSEF, 2010). The second most important application is in polymer dispersion as textile back-coating on cotton or cotton mixed with synthetic blends. In this application HBCDDs particles need to be very small. The material therefore needs to be micronised. During 2002 to 2004 some 1,000 tonnes per year of HBCDD was micronised. According to information from the textile industry the use of HBCDD in textile applications has decreased by 80 % until 2008 and, therefore, probably also the production of micronised HBCDD. The concentration of HBCDD in the back-coating of textiles could range from 2.2-4.3 % (Kajiwara et al., 2009).

A further smaller application of HBCDDs is in HIPS (high impact polystyrene) which is used in electrical and electronic equipment and appliances at concentrations between 1 and 7 % (ECHA, 2009a). HBCDDs may also be added to latex, adhesives and paints (Albemarle Corporation, 2000; Great Lakes Chemical Corporation, 2005). The use of HBCDDs in EPS in packaging material is believed to be of minor importance and HBCDDs are not used in food packaging materials (ECHA, 2009a). According to a submission from Germany to the Stockholm convention, HBCDDs are used in EPS filling in e.g. nursing pillows and bean bags used as easy chairs. Granulated EPS waste is used to improve the texture of agricultural and horticultural soil.

4.3. HBCDDs in the environment

4.3.1. Release into the environment

The total release of HBCDDs to the environment is increasing in the EU, in spite of the recent decrease in the releases from textile back-coating (ECHA, 2009a). The total releases of HBCDDs from manufacture and use of insulation boards and manufacture and use of textiles are in the same magnitude. Total releases from manufacture and use of electronic devices are minor. According to the substance-flow analysis done in Switzerland (Morf et al., 2008) construction materials are responsible for the majority of the releases. In Switzerland the release during the service life of products was found to be the dominating source of HBCDDs (Morf et al., 2008), whereas the releases from industrial point sources were dominating in the analysis performed in the EU (ECHA, 2009a).

Säll (2010) has compiled estimated releases of HBCDDs in EU27 to the environment from manufacture and different uses of HBCDDs based on figures for 2006 from the HBCDD Industry Users Group. Data on textile coatings refer to 2007 and are taken from ECHA (2009a). The estimated total release to the environment is 3,140 kg per year, whereof 2,560 kg originate from point sources. These figures contain a lot of uncertainty and they should be regarded to be low estimates as there are a number of routes of release that have not been included in this estimation. It is however interesting to notice that the production and use in textiles give rise to almost 50 % of the total estimated release despite that the main use of HBCDDs in polystyrene foam attributes around 80 % of the total use. It is likely that the contribution from polystyrene foam will increase in the future as more and more of this material will become waste.

4.3.2. Transformation in the environment

Davis et al. (2005) studied the degradation of technical HBCDD in freshwater sediments and soils and found that, in both media, the rate of loss at 20 °C was appreciably faster under anoxic conditions. Using sterile controls, biotransformation of HBCDDs was found to be faster in the presence of

microorganisms and half-lives ranged from 11 to 32 days (aerobic) and 1.1 to 1.5 days (anaerobic) in sediment. In soil, half-lives under aerobic and anaerobic conditions were 63 and 6.9 days, respectively. It should be noted that in this study only the degradation of γ-HBCDD was studied since the levels of both α- and β-HBCDD were below the limit of detection (LOD).

In the ECB risk assessment report (ECB, 2008), the degradation half-lives of HBCDDs in aerobic sediment at 20 °C were calculated to be 113, 68 and 104 days for α-, β- and γ-HBCDD, respectively. In sediment, technical HBCDD was observed to be subject to degradation with half-lives of 11-32 and 1.1-1.5 days in anaerobic and aerobic river sediment when tested according to OECD Test Guidelines (Davis et al., 2005). The corresponding half-lives in soil, when tested according to OECD Test Guideline 307, were 63 and 6.9 days (Davis et al., 2005). No brominated transformation products were indicated. However, in a subsequent study, three debrominated products were identified as tetrabromocyclododecene, dibromocyclododecadiene and cyclododecatriene, indicating stepwise vicinal debromination.

HBCDDs can not undergo hydrolysis but elimination reactions may occur in water. According to calculations in the EMEP report on HBCDDs (EMEP, 2009), the physical-chemical properties of the technical mixture and γ-HBCDD stereoisomer give a half-life in water of about 5 years. According to the European Brominated Flame Retardants Industry (EBFRIP, 2009) the half-life in water and soil derived from comparing different model estimations lies in the range of 8.5 to 850 days. HBCDDs will most likely undergo reductive debromination also under abiotic conditions. Since HBCDD is not absorbing UV light above 290 nm direct photolysis is not possible but since it is shown that HBCDDs are undergoing photolysis (Harrad et al., 2009a) this must occur through indirect photolysis.

4.3.3. Occurrence in the environment

Total HBCDDs can be found as widespread contaminants in the global environment, with high levels in the top predators. According to Covaci et al. (2006) high concentrations have been measured in marine mammals and birds of prey. According to recent reviews, the levels of HBCDDs in the environment are generally increasing in all matrices studied and they seem to correlate with the increasing use of HBCDDs (UNEP, 2010).

4.3.3.1. Air and dust

Two reviews dealing with the occurrence of HBCDDs in air and dust (Covaci et al. 2006; Law et al., 2008) have been identified. HBCDDs have been found in Arctic air (Svalbard) at mean concentrations of 7.1 pg/m^3 (2006) and 6.5 pg/m^3 (2007). γ-HBCDD was the predominant stereoisomer followed by α-HBCDD and a very low contribution from β-HBCDD. Earlier results from more or less remote stations in Sweden and Finland range from 2 to 280 pg/m^3, whereas the mean concentration in ambient air at a production site is reported to be 280 ng/m^3.

Levels of indoor air at sites for production of HBCDDs (bag filling and reactor sites) range from 9,400 to 28,500 ng/m^3 and dust from sites for production of expanded polystyrene are reported to contain <13-1,600 µg/m^3. House dust from homes and offices in Europe are reported to contain <3 (LOD) to 58,000 ng/g and the mean concentrations in offices and homes under study in Belgium and UK were 4,800 and 3,160 ng/g, respectively. Generally, in North America, the corresponding levels in air and dust were found to be lower than in Europe.

HBCDDs are persistent in air, with an estimated half-life of more than two days. Considering the uncertainty in model estimates, it has been estimated that the range could be 0.4 to 4 days and 0.6 to 5.4 days for the northern and southern hemisphere, respectively (EBFRIP, 2009). Studies on the modelling of the environmental fate and transport of HBCDDs, as well as field data including information on levels of HBCDDs in the Arctic atmosphere, provide evidence of the potential for long-range transport of HBCDDs (UNEP, 2010).

In a study by Abdallah et al. (2008b), HBCDDs were determined in indoor air from 33 UK homes 25 offices, 4 public microenvironments (i.e. pubs and restaurants) as well as in 5 outdoor air samples. The mean concentration of the sum of HBCDDs was found to be 250, 180, 900 and 37 pg/m^3, respectively. In the same study the mean concentration of the sum of HBCDDs in dust samples from 45 homes, 28 offices, 20 cars and 4 public microenvironments was found to be 8,300, 1,600, 19,000 and 2,700 ng/g, respectively. The composition of HBCDDs in dust was on average found to be 33 % α-, 11 % β-, and 56 % γ-HBCDD, while the corresponding fractions in air were 22 % α-, 11 % β- and 65 % γ-HBCDD.

Harrad et al. (2010) studied the occurrence of BFRs and other POPs in dust in samples (n=43) collected during winter 2007 in day-care centres and primary schools in the West Midlands of the UK. The concentrations of HBCDDs were significantly higher than same group previously reported from homes and offices. The median concentration in dust was found to be 4,100 ng/g with a range of 72 to 89,000 ng/g. The median concentrations of α-, β- and γ-HBCDD in the same samples were 1,400, 550 and 1,700 ng/g, respectively.

Harrad and Abdallah (2011) studied the occurrence of α-, β- and γ-HBCDD but also tetra- and pentabromocyclododecanes (sum of tetraBCDs and sum of pentaBCDs) in dust collected in 14 car cabins. The median concentrations of the sum of HBCDDs ranged from 1,200 to 9,800 ng/g dust. The median concentrations of α-, β- and γ-HBCDD were found to be 3,000, 1,100 and 4,800 ng/g, respectively. The authors also studied HBCDDs in trunk dust and found that α-HBCDD was significantly more prevalent in cabin dust than in trunk dust (33 % vs. 22 %). For γ-HBCDD, the result was the opposite, 55 % in cabin dust and 68 % in trunk dust. The relative occurrence of β-HBCDD was found to practically identical in cabin and trunk dust (11 and 10 %, respectively). The shift towards α-HBCDD in cabin dust could be interpreted as a result of photolytic isomerisation from γ-HBCDD to α-HBCDD. This is supported by the observations in experimental studies by Harrad et al. (2009a). The median concentrations of the sum of tetraBCDs and pentaBCDs were found to be much lower: 15.5 and 81 ng/g, respectively.

4.3.3.2. Soil and uptake by plants

HBCDDs in soil have been studied by Covaci et al. (2009). The concentrations were < LOD-6.6 ng/g dry weight (d.w.), median 0.18 ng/g d.w. No significant correlation was found between concentrations of sum PBDEs and HBCDDs in the soil samples.

Schlabach et al. (2002) detected α-HBCDD in 6 out of 10 moss samples (*Hylocomium splendens*), and γ-HBCDD in 2 out of 10 samples. The highest concentration, about 11 mg HBCDD/kg wet weight (w.w.), was detected in a moss sample from a monitoring station at the Norwegian south-southwest coast. The detected concentrations decreased further north along the Norwegian coastline, from 586 and 291, to below the LOD of 1.5 µg HBCDD/kg w.w. As mosses lack the root system function present in vascular plants these results do not reflect plant uptake from soil but rather the impact from atmospheric deposition. In the only identified report on root uptake of HBCDDs (Li et al., 2011) seeds of cabbage and radish were grown in soil containing 1,000 µg HBCDDs/kg. The uptake of HBCDDs via the roots was determined. The results show that after 8 weeks cabbage can contain up to 65 µg/kg in root tissue and 30 µg/kg in shoot tissue. The same authors also found that the root tissue contained higher levels of γ-HBCDD compared to α- and β-HBCDD, up to around 40, 18 and 12 µg/kg, respectively, whereas in the shoots α-HBCDD was the dominating isomer followed by γ- and β-HBCDD with levels up to around 18, 8 and 3 µg/kg, respectively.

4.3.4. Bioaccumulation and biomagnification in wildlife

There are only a few studies of bioaccumulation and biomagnification in wildlife.

Veith et al. (1979) calculated a bioconcentration factor (BCF) at steady-state of 18,100 in fathead minnow (*Pimephales promelas*) in a 32-day flow-through test. The mean test concentration of HBCDDs was 6.2 µg/L and test temperature 25 ± 0.5ºC. Drottar and Krueger (2000) calculated a BCF at a nominal concentration of 3.4 µg HBCDD/L in Rainbow trout (*Onchorhynchus mykiss)* (whole fish) to be 8,974. This was further specified as 4,850 in edible tissue and 12,866 in non-edible tissue. Based on this study and the one by Veith et al. (1979), an overall BCF for aquatic organisms of 18,100 was chosen in the EU risk assessment (ECB, 2008).

The biomagnification factor (BMF) describes the concentration change that can occur with the trophic level in an ascending (aquatic) food chain, e.g. from micro invertebrates as small crustaceans via fish to fish eating birds and seals. Studies of a marine food web containing mussels, polychaetes, crabs and seabird eggs revealed that the accumulation of HBCDD did not correlate well with lipid content for most of the species (Haukås et al., 2010). In contrast to β- and γ-HBCDD, α-HBCDD increased significantly with the trophic level, resulting in biomagnification factors above 1 in this coastal marine ecosystem.

Law et al. (2006) studied the BMF of individual HBCDD stereoisomers in juvenile rainbow trout (*Oncorhynchus mykiss)* fed with feed containing α-, β- or γ-stereoisomer. The authors were able to calculate BMFs of 9.2, 4.3 and 7.2 for α-, β- and γ-stereoisomers, respectively. The BMFs were derived by multiplying the assimilation efficiency with the feeding rate and then divide this product by the depuration rate constant. Interestingly they also noted that bio-isomerisation, i.e. conversion of one isomer into another, can occur *in vivo* in at least this fish species. It was found that after termination of the biomagnification test (day 168), in muscle samples of fish exposed solely to β-HBCDD a major part was in the form of α- and γ-HBCDD. In the fish exposed to α-HBCDD, no shift to other stereoisomers was found.

A temporal trend study of HBCDDs in ringed seals (*Pusa hispida*) from east Greenland show increasing trend with present levels just below 10 ng/g fat (Vorkamp et al., 2011). It has not been possible to identify any corresponding field studies in the terrestrial environment, but two laboratory studies show that HBCDDs have a potential to bioaccumulate in terrestrial mammals.

4.4. Combustion

The combustion of domestic products containing common BFRs such as HBCDDs may lead to concurrent emissions of HBCDDs and polybrominated dibenzo-*p*-dioxins and furans (PBDD/Fs). Large amounts of brominated and mixed chloro-bromodioxins and furans can be formed in accidental fires where brominated flame retardants are present (Söderström and Marklund, 1999; Lundstedt, 2009). Thus, incineration of materials containing HBCDDs can, especially when carried out under uncontrolled conditions, lead to substantial emissions of a variety of hazardous substances.

5. Occurrence and patterns of HBCDDs in food

5.1. Current occurrence of HBCDDs in food: call for data

Following a European Commission request, in 2005 the CONTAM Panel (EFSA, 2006) identified HBCDDs as an important BFR to be monitored. From October 2006, EU-wide monitoring of BFRs including HBCDDs was organised and the results of this exercise were made available to EFSA. In addition, a call for data on BFRs[11] from the Dietary and chemical Monitoring Unit (DCM) (former

[11] http://www.efsa.europa.eu/en/data/call/datex091215.htm

Data Collection and Exposure Unit, DATEX) was issued by EFSA in December 2009, with different deadlines according to the chemicals to be collected. The closing date for data submissions on HBCDDs was July 2010.

EFSA collected and evaluated the results reported from the analysis of 1,914 food samples. Data were provided by seven European countries and covered the period from 2000 and 2010.

The data submission to EFSA followed the requirements of the Standard Sample Description model. On the EFSA webpage of the call for data detailed instructions on how to submit data[12] and the Guidance on Standard Sample Description for Food and Feed[13] specifying the data elements, the sample data structure of the analytical results for chemical contaminants and residues in food and feed were provided.

SAS Enterprise software was used to extract information from the occurrence data submitted. Data providers were asked to check and eventually confirm that the extracted information were correct and provide clarifications in case of unclear or missing detailed information

5.1.1. Summary of data collected

The origin of the 1,914 samples reported from the seven European countries is illustrated in Figure 3. Norway provided 47 % of the data followed by France (14 %), Great Britain (14 %) and Sweden (13 %).

The distribution of results over the years of sampling is illustrated in Figure 4. Almost 80 % of the samples were analysed in the years 2006 to 2009. The year 2010 was not a complete year of sampling, as the closing date of the call for data for HBCDDs was July 2010.

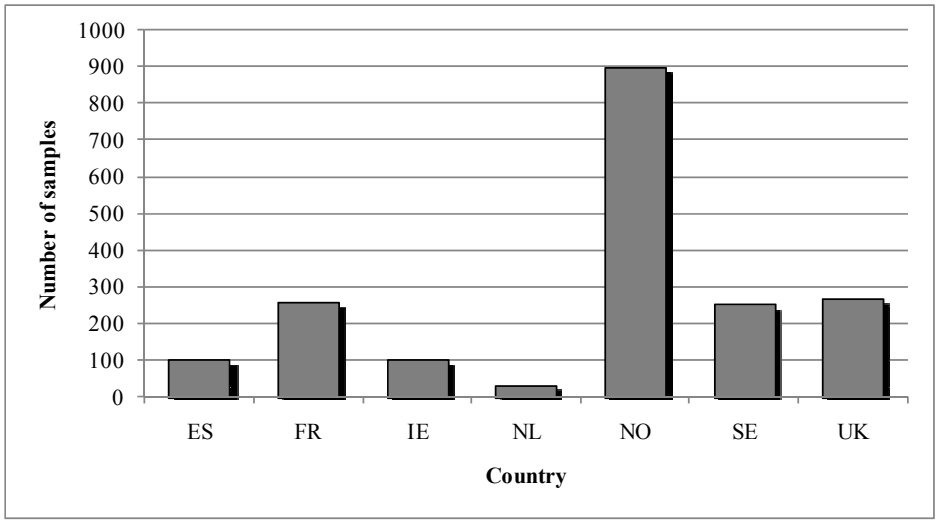

Figure 3: Distribution of samples across seven European countries (ES: Spain, FR: France, UK: United Kingdom, IE: Ireland, NL: Netherlands NO: Norway, SE: Sweden).

[12] http://www.efsa.europa.eu/en/datexcallsfordata/datexsubmitdata.htm
[13] http://www.efsa.europa.eu/en/scdocs/scdoc/1457.htm

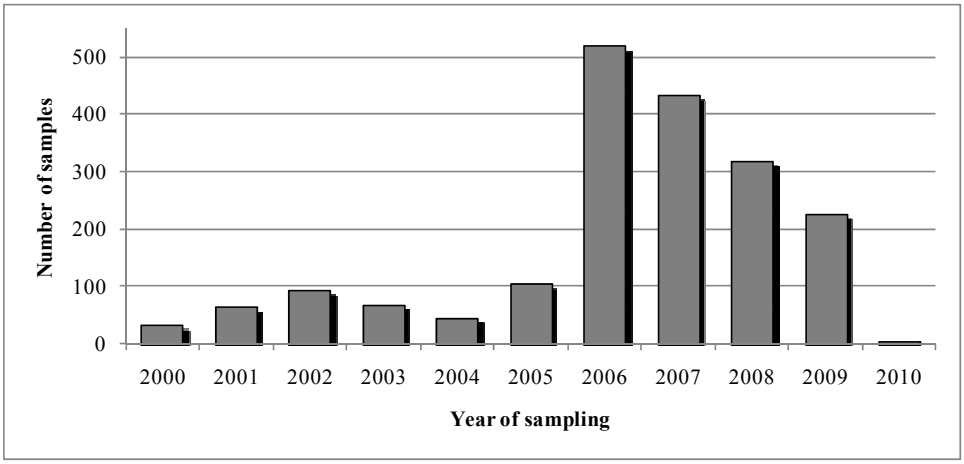

Figure 4: Distribution of samples over the years of sampling.

Analytical results identified during the data cleaning steps with incomplete or incorrect description of any of the required variables (e.g. parameter type, food classification, results value or results limit of detection (LOD)-limit of quantification (LOQ) of the Standard Sample Description template (EFSA, 2010), were returned to the respective data provider for further check, before excluding the records from the database.

Additionally, specific exclusions were performed concerning data from total diet studies (TDS) and from targeted surveys for a total of 61 samples. For the targeted surveys this was done because they could influence the overall occurrence values; for the TDS samples, because of not correct matching between the reported food descriptions of the TDS pooled composite food with the food categories of the current FoodEx food classification system.

Additionally, in view of a comprehensive occurrence description and dietary exposure assessment of α-, β-, γ-HBCDD and total HBCDD, eight samples were discarded because the information on the three stereoisomers was not complete.

Furthermore, 20 samples where α-, β-, γ-HBCDD stereoisomers were analysed individually were excluded because of the uncertainty linked to the analytical methods used. The data provider explained that those results were not suitable for dietary exposure assessment purposes.

With final consent of the respective data providers, a total of 3,776 analytical results covering α-, β-, γ-HBCDD and total HBCDD (from 1,825 samples) were included in the HBCDD dataset for the calculation of the dietary intake.

5.1.2. Distribution of analytical results reported for HBCDDs

A total of 1,825 samples were tested for α-, β-, γ-HBCDD and total HBCDD and a total of 3,776 analytical results were collected. In certain cases in one food sample only the presence of the individual stereoisomers α-, β-, γ-HBCDD was tested. In other cases the individual stereoisomers and the mixture of the total HBCDD were analysed or only total HBCDD were measured. Therefore, in Table 3, the pattern of analysis of the three stereoisomers or total HBCDD is presented across the number of samples. A number of 997 analytical results were reported for each individual stereoisomer, whereas 995 analytical results were reported for total HBCDD.

Table 3: Pattern of analysis of total HBCDD and α-, β- and γ-HBCDD (individual stereoisomers) across the number of samples.

Pattern of stereoisomers and total HBCDD reported	Number of samples
α-, β-, γ-HBCDD (individual stereoisomers)	830
Total HBCDD and α-, β-, γ-HBCDD (individual stereoisomers)	97
Total HBCDD	898

5.1.3. Distribution of samples reported for food groups

Data providers were asked to codify all food descriptors according to the food classification system of EFSA Concise European Food Consumption Database (EFSA concise food categories).[14]

In order to improve the estimation of the dietary exposure assessment, the 'Comprehensive European Food Consumption Database' was established in 2010 with a refined food classification named FoodEx.

FoodEx is a food classification system developed by EFSA's DCM Unit in 2009 with the objective to simplify the link between occurrence and food consumption data when assessing the dietary exposure to hazardous substances. FoodEx contains 20 main food groups (first level),[15] which are further divided into subgroups having 160 items at the second level, 1,261 items at the third level and about 1,800 end-points (food names or generic food names) at the fourth level. It is based on a hierarchical coding for easy cross-checking and it is structured as a child-parent relation. The distribution of the 1,825 samples across the aggregated food groups is shown in Figure 5.

For HBCDDs the food group of 'Fish and other seafood (including amphibians, reptiles, snails and insects)' dominated the product coverage with 71 % of the total samples, followed by 'Meat and meat products (including edible offal)' and 'Eggs and egg products', at 10 % and 7 % respectively, and 'Milk and dairy products' at 5 %.

For the food groups of 'Animal and vegetable fats and oils', 'Products for special nutritional use' and 'Food for infants and small children' respectively 80, 16 and 10 samples were analysed for the presence of HBCDDs. Less than 9 samples were reported for the remaining food groups.

From the 20 aggregated food groups available in the first level of FoodEx, only 12 of them were covered in the current data collection. No analytical results for food products in the groups of 'Legumes, nuts and oilseeds', 'Sugar and confectionary', 'Fruit and vegetable juices', 'Non-alcoholic beverages (excepting milk based beverages)', 'Alcoholic beverages', 'Drinking water (water without any additives except carbon dioxide; includes water ice for consumption)', 'Herbs, spices and condiments' and 'Snacks, desserts' were submitted to EFSA.

[14] http://www.efsa.europa.eu/en/datex/datexfooddb.htm

[15] Grains and grain-based products, Vegetables and vegetable products (including fungi), Starchy roots and tubers, Legumes, nuts and oilseeds, Fruit and fruit products, Meat and meat products (including edible offal), Fish and other seafood (including amphibians, reptiles, snails and insects), Milk and dairy products, Eggs and egg products, Sugar and confectionary, Animal and vegetable fats and oils, Fruit and vegetable juices, Non-alcoholic beverages (excepting milk based beverages), Alcoholic beverages, Drinking water (water without any additives except carbon dioxide; includes water ice for consumption), Herbs, spices and condiments, Food for infants and small children, Products for special nutritional use, Composite food (including frozen products), Snacks, desserts, classification not possible.

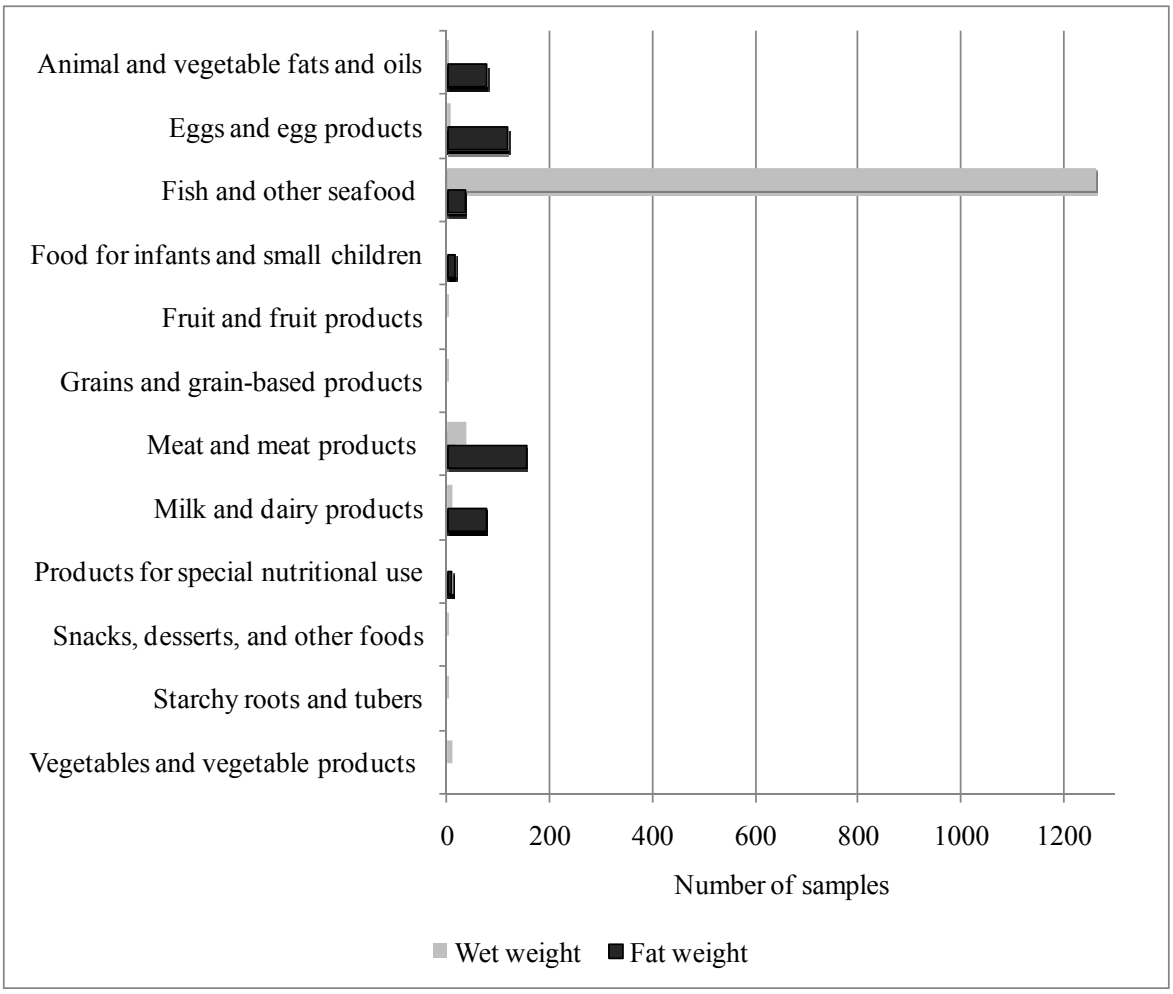

Figure 5: Distribution of samples in the aggregated FoodEx food groups (first level) according to the respective expression of results (wet weight, fat weight).

The analytical results of products of animal origin, excluding fish, were mainly reported on a fat basis (81-100 % of the cases). For 'Fish and other seafood' only in 3 % of the samples the results were expressed on a fat basis.

For estimating the dietary exposure to HBCDDs, all analytical concentrations expressed on fat weight needed to be converted to whole weight basis (wet weight). For this reason the data providers were requested to report the fat content in the Standard Sample Description template for each analysed sample. Only one case, a 'Whole egg, chicken' sample (FoodEx third level) was reported without the original fat content. In this case the missing information was replaced by the average fat content calculated in the Comprehensive Food Consumption Database (10.5 %).

5.1.4. Analytical methods used and limits of detection

The analytical methods used to perform the analyses of individual HBCDD stereoisomers are mainly based on LC-MS/MS. In particular, 49 % and 36 % of the results were obtained by LC-MS/MS and high performance liquid chromatography (HPLC)-MS/MS, respectively. The remaining 15 % of the analytical results were obtained by LC-MS. On the contrary, GC-MS was the predominant method for the analysis of total HBCDD (85 %). The remaining 15 % of the results of total HBCDD were reported with GC-ECD.

According to the specific requirements of the call for data (DCM call for data on BFRs[6]), the analytical results should have been reported accompanied with the percentage of recovery. Most of data were correctly reported. Therefore, in order to harmonise the database, the correction for recovery was applied where needed. In some cases the analytical results were reported as not corrected by recovery but the recovery rate was not provided. In those cases, due to the possible uncertainty associated with imputed recovery values, no additional correction was applied and the results were considered as corrected for recovery.

The submitted analytical results have been reported in different units. For the sake of comparison all measurements have been converted into ng/g w.w.

The LOD and LOQ for the analyses could vary with the individual stereoisomers or total HBCDD under analysis (Figure 6), the analytical technique, the food matrix and the analysing laboratory. In Figure 6, the box indicates 25th and 75th percentile with a line at the median, and the ends of the whiskers represent the 5th and 95th percentiles. In order to compare the values reported for LOQs across stereoisomers and total HBCDD and across different food matrices, all the LOQs values have been expressed on w.w.

The lowest LOQs were reported for the β-HBCDD with median of 0.01 ng/g w.w., while the highest results were reported for α-HBCDD with median of LOQs of 0.08 ng/g w.w.

The spread of the LOQs reported by individual congeners and total HBCDD takes into account the variability of the different HBCDD stereoisomers specific LOQs within each food group and analytical method. In particular, the highest LOQs were reported in the food group of 'Fish and other seafood' with medians of 0.53, 0.16, 0.09 and 1.15 ng/g w.w., for α-, β-, γ-HBCDD individual stereoisomers and total HBCDD, respectively (data not reported).

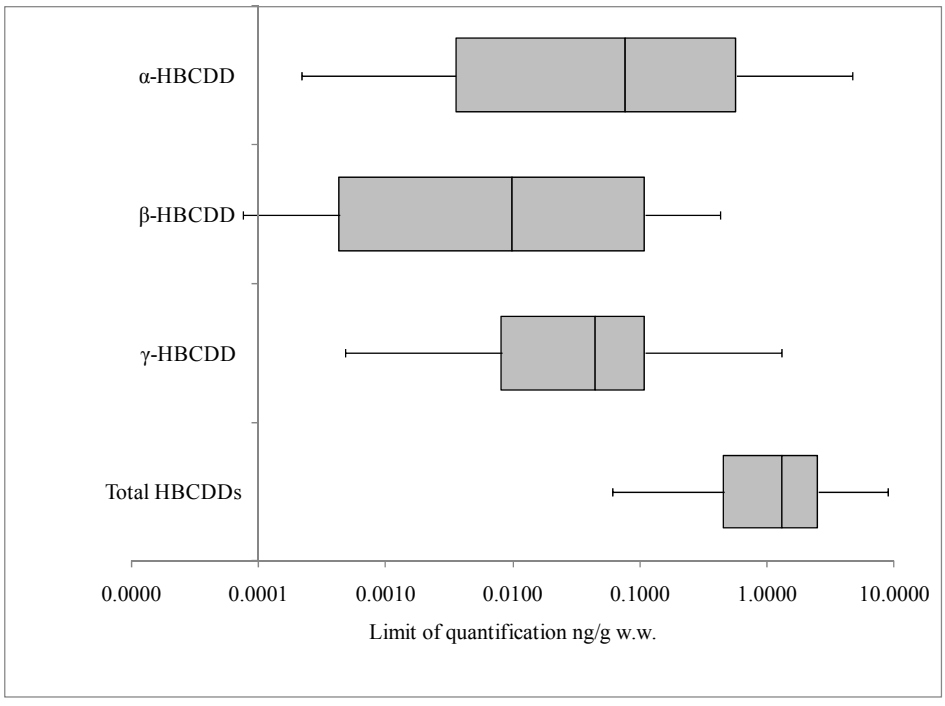

Figure 6: Distribution of the limits of quantification (LOQ) for the individual HBCDD stereoisomers and total HBCDD.

5.1.5. Occurrence data by food group

The number of analytical records reported as quantified values was 23 % out of 3,776 results across individual α-, β-, γ-HBCDD stereoisomers and total HBCDD, with no quantified results for the food groups of 'Snacks, desserts, and other foods' and 'Starchy roots and tubers'. For the food groups 'Milk and dairy products', 'Fish and other seafood (including amphibians, reptiles, snails and insects)' and 'Eggs and egg products' the percentage of reported quantified results was 19, 25 and 26 %, respectively (Figure 7).

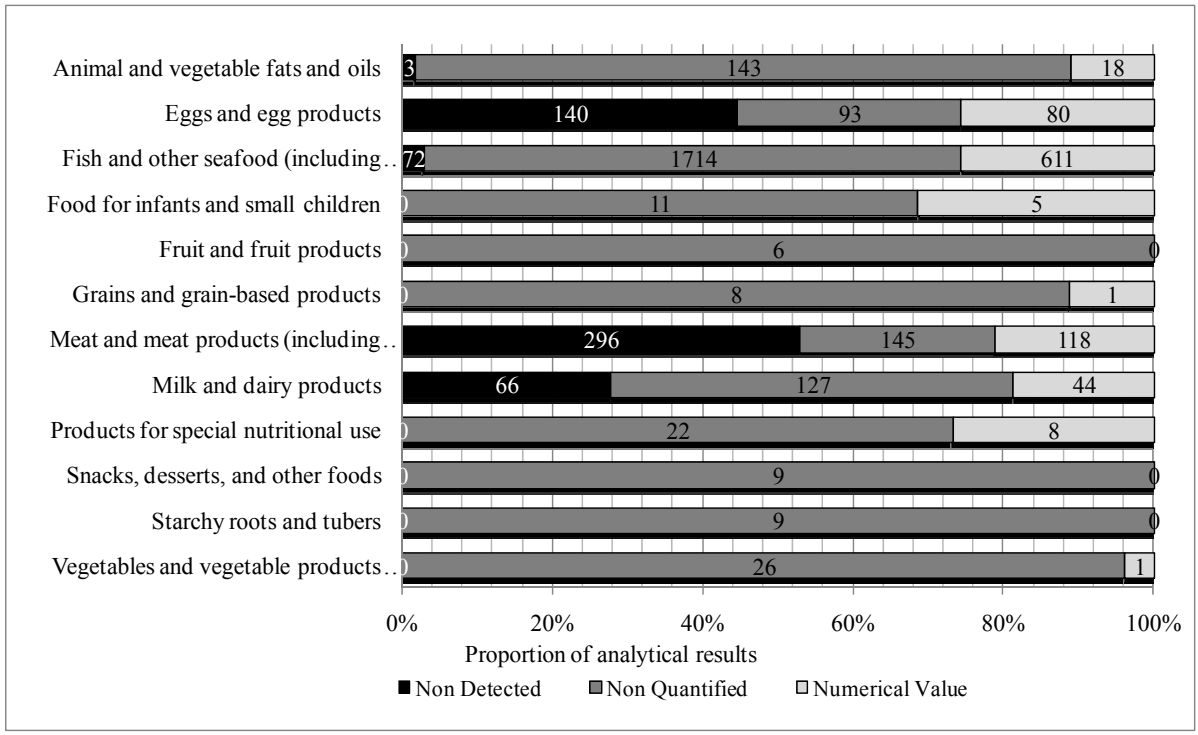

Figure 7: Proportion of non detected, non quantified and quantified analytical results across food categories in the first level (broad food groups) of the FoodEx classification system.

Similarly, the number of quantified results was inspected for α-, β- and γ-HBCDD and total HBCDD (Table 4). The criteria for handling left-censored data which are described in a recently published EFSA report (EFSA, 2010) were applied as a screening tool to the HBCDDs database, separately for each combination of HBCDDs compound (α-, β- and γ-HBCDD and total HBCDD) and aggregated food group (first level of the FoodEx system). These criteria offer guidelines to apply statistical method to left censored data when the sample size is greater than 50 observations (or there are more than 25 positive samples), and when the percentage of non-detects is less than 80 %. In the event in which these criteria are not met, it is recommended to pool similar food groups to obtain larger sample sizes, or to collect additional data. In the current document the term non-detects refers to results either below LOD or below LOQ, depending on which limit was reported.

Table 4: Frequency of results (N) and proportion of non-detects (ND %) values across α-, β- and γ-HBCDD and total HBCDD and food group of the FoodEx food classification system. The column ND (%) indicates the percentage of results below the LOD or the LOQ. The results are estimated from 3,776 analytical results obtained by the analysis of 1,825 samples. If no data are given in the table ('-'), it means that no analytical results were available. In bold are highlighted the food groups that passed the screening exercise on data quality.

	HBCDD compounds							
	α- HBCDD		β-HBCDD		γ-HBCDD		Total HBCDD	
Food groups (FoodEx_Level 1)	**N**	**ND (%)***	**N**	**ND (%)***	**N**	**ND (%)***	**N**	**ND (%)***
Animal and vegetable fats and oils	42	100 %	42	100 %	42	100 %	38	53 %
Eggs and egg products	94	53 %	94	87 %	94	93 %	31	45 %
Fish and other seafood	501	71 %	501	90 %	501	87 %	894	61 %
Food for infants and small children	-	-	-	-	-	-	16	69 %
Fruit and fruit products	2	100 %	2	100 %	2	100 %	-	-
Grains and grain-based products	3	100 %	3	100 %	3	67 %	-	-
Meat and meat products	184	58 %	184	88 %	184	90 %	7	100 %
Milk and dairy products	76	74 %	76	74 %	76	96 %	9	89 %
Products for special nutritional use	10	60 %	10	80 %	10	80 %	-	-
Snacks, desserts, and other foods	3	100 %	3	100 %	3	100 %	-	-
Starchy roots and tubers	3	100 %	3	100 %	3	100 %	-	-
Vegetables and vegetable products	9	89 %	9	100 %	9	100 %	-	-

*The column ND (%) indicates the percentage of results below the LOD or the LOQ.

The results of this screening exercise on the HBCDDs data show that only the broad food groups of 'Fish and other seafood', 'Meat and meat products', 'Milk and dairy products', 'Eggs and egg products' meet the statistical criteria set in the EFSA report, for at least one of the HBCDD compounds (stereoisomers and total HBCDD). This finding is supported by literature data, which overall shows substantial evidence of HBCDDs contamination in food groups of animal origin (see Chapter 6) and especially in fish, due to the lipophilic nature of those compounds. Based on these observations, the CONTAM Panel decided to focus the dietary exposure estimation for the HBCDDs from a restricted list of food groups: 'Fish and other seafood', 'Meat and meat products', 'Milk and dairy products', 'Eggs and egg products'. The screening exercise was not applied to the food group 'Products for special nutritional use' and 'Food for infants and small children', as a separate intake estimation will be carried out for the specific group of the population consuming food supplements.

Despite the high number of non-detects, the evaluation of the left-censored data in the HBCDDs database was performed in accordance with the guidelines of the WHO-Global Environment Monitoring System-Food Contamination Monitoring and Assessment Programme (GEMS/Food). As mentioned in the guidance the lower and upper bounds approach should be used when the quantified results are below 40 % (WHO, 2003). The lower bound (LB) is obtained by assigning a value of zero (minimum possible value) to all samples reported as lower than the LOD (<LOD) or LOQ (<LOQ). The upper bound (UB) is obtained by assigning the numerical value of LOD to values reported as <LOD, and LOQ to values reported as <LOQ (maximum possible value), depending on whether LOD or LOQ is reported by the laboratory.

All the food samples were classified according to the FoodEx classification system. The spread of the analytical results across the several FoodEx groups and the high percentage of not detected or not quantified analytical results prevented from calculating summary statistics at a very detailed level of the food classification system.

Table 5 reports a description of contamination values for α-, β- and γ-HBCDD and total HBCDD (where available). Additionally the sum of the three stereoisomers was calculated and reported. Contamination levels are given on the mean occurrence of the different HBCDD compounds for the broad food groups in the first level of the FoodEx. The column ND (%) indicates the percentage of results below the LOD or the LOQ. The mean fat content (%) calculated on the reported original samples is also reported.

Due to the selection of food groups for the purpose of dietary exposure assessment the database has been restricted to 1,725 food samples (3,552 analytical results).

Eggs and eggs products

The food group 'Eggs and eggs products' consists of 125 samples. For 94 samples, analytical results on the individual stereoisomers were reported and for the remaining 31 samples, results on total HBCDD were submitted.

The LB and UB mean of the sum of the three stereoisomers estimated from 94 samples is 0.14 and 0.54 ng/g fat, respectively. The calculated sum of the three stereoisomers is higher than the LB and UB mean for total HBCDD as measured (0.15 and 0.20 ng/g fat) and the major contributing isomer to the sum is α- HBCDDs.

Milk and dairy products

Within the food group 'Milk and dairy products' a total of 85 samples were analysed; in 76 samples the three individual congeners was measured, while in only 9 samples the total HBCDD was analysed. Among the 'Milk and dairy products', 77 samples were described as 'Liquid milk' and 8 as 'Cheese' (FoodEx level 2; data not reported). Overall, the proportion of non-detects is higher for γ-HBCDD with 96 % non-detects, followed by α- and β- HBCDDs with 74 % non-detects. Probably due to the influence of the non-detects, the difference between the LB and UB mean estimates is higher for γ-HBCDD (0.0002 and 0.29 ng/g fat, respectively) compared with the other two stereoisomers. The LB and UB overall mean (estimated on 76 samples) of the sum of the three stereoisomers reaches 0.03 and 0.67 ng/g fat, respectively. The LB and UB mean for total HBCDD as measured is lower than the calculated sum (0.02 and 0.15 ng/g fat).

Table 5: Statistical description of mean concentrations of α-, β- and γ-HBCDD, the calculated sum of individual stereoisomers and total HBCDD, across five broad food groups of the FoodEx food classification system. HBCDD concentrations are reported on a fat (ng/g fat) or wet weight basis (ng/g w.w.) according to the different food groups. The number of analytical results (n) and proportion of non-detects (ND %) across HBCDDs compounds and food groups and mean fat content calculated from the original samples (%) is also reported.

LB: lower bound; UB: upper bound.

		HBCDD compounds															
		α-HBCDD			β-HBCDD			γ-HBCDD			HBCDD-sum (α+β+γ)			Total-HBCDDs			Mean percentage of fat in the original sample (%)
Food groups (FoodEx_Level 1)	**TYPE**	**n**	**MEAN**	**ND (%)[a]**	**n**	**MEAN**	**ND (%)[a]**	**n**	**MEAN**	**ND (%)[a]**	**n**	**MEAN**	**ND (%)[a]**	**n**	**MEAN**	**ND (%)[a]**	
Results expressed on a fat basis (ng/g fat)																	
Eggs and egg products	LB	94	0.13	53	94	<0.01	87	94	0.01	93	94	0.14		31	0.15	45	11.29
	UB	94	0.28	53	94	0.07	87	94	0.19	93	94	0.54		31	0.20	45	11.29
Milk and dairy products	LB	76	0.03	74	76	<0.01	74	76	<0.01	96	76	0.03		9	0.02	89	5.64
	UB	76	0.23	74	76	0.16	74	76	0.29	96	76	0.67		9	0.15	89	5.64
Meat and meat products (including edible offal)	LB	184	0.11	58	184	0.01	88	184	0.02	90	184	0.14		7	0.00	100	4.45
	UB	184	0.31	58	184	0.19	88	184	0.29	90	184	0.79		7	0.38	100	4.45
Results expressed on a wet weight basis (ng/g w.w.)																	
Products for special nutritional use	LB	10	1.06	60	10	0.07	80	10	0.08	80	10	1.21	.	.	.	.	100.00
	UB	10	1.37	60	10	0.24	80	10	0.25	80	10	1.86	.	.	.	.	100.00
Food for infants and small children	LB	.	.	.	.	.	.	.	.	.	.	.	.	16	0.01	69	7.24
	UB	.	.	.	.	.	.	.	.	.	.	.	.	16	0.03	69	7.24

(a): The column ND (%) indicates the percentage of results below the LOD or the LOQ.

Meat and meat products (including edible offal)

In the food group 'Meat and meat products (including edible offal, ng/g fat)' a total of 191 samples were analysed. In particular 184 samples were analysed for the individual HBCDDs stereoisomers and only 7 for the presence of total HBCDD. In this food group, food classified as sausages (31 %), edible offal from game and farmed mammals (29 %), livestock meat (23 %), poultry (12 %) and meat from game mammals (5 %), were included (FoodEx level 2, data not reported).

The proportion of not detected or not quantified results varies from 58 % for α-HBCDD to 88 % for β-HBCDD and 90 % for total HBCDD. In addition to the lower number of non-detects, α-HBCDD is the stereoisomer contributing more to the overall occurrence of HBCDDs in this food group. The LB and UB occurrence means in the 'Meat and meat products (including edible offal)' food group for the sum of the three HBCDDs stereoisomers reaches respectively 0.14 and 0.79 ng/g fat (Table 5), and no major differences in contributions were observed at a more disaggregated level of the food classification (FoodEx level 2).

Food for infants and small children

The food group 'Food for infants and small children' consists of 16 samples for which only total HBCDD was analysed. Out of these 16 samples, 3 were described as 'Infant formulae, powder' and the other 13 samples were reported as 'Ready-to-eat meal for infants and small children' with respectively a proportion of non-detects of 100 % and 62 % (FoodEx level 2; data not reported). No major differences in the LB and UB mean occurrence estimation were observed among infant formulae and ready-to-eat meal, therefore an overall LB and UB mean estimation was done. The LB and UB occurrence means for total HBCDD in the overall group is 0.01 and 0.03 ng/g w.w., respectively.

Products for special nutritional uses

Within the food group 'Products for special nutritional uses' in a total of 10 samples the three individual HBCDD stereoisomers were analysed. All those samples were described more in details as 'Supplements containing special fatty acids (e.g. omega-3, essential fatty acids)' (FoodEx level 3; data not reported). The LB and UB mean of the sum of the three stereoisomers is 1.21 and 1.86 ng/g fat and it is highly influenced by α-HBCDD. The proportion of non-detects for α-HBCDD is lower than for the other two stereoisomers (6 out of 10 samples) and the LB and UB mean estimation is higher, reaching up to 1.06 and 1.37 ng/g fat. Moreover, details were reported describing the form on how the fish oil was sold. Three samples were described as fish oil bottled and seven as fish oil in capsules. The fish oil supplement in its liquid form contains up to ten times higher levels of α-HBCDD than the capsules (up to 2.83 and 2.99 ng/g w.w. for LB and UB mean, respectively).

Fish and fish products

The food group of fish and fish products is the most populated group with 1,298 samples. Fish meat samples cover the 69 % of the data, followed by water molluscs (15 %), other fish offal described as fish liver (14 %), crustaceous (3 %), fish roe (four samples) and fish products (one sample). Details on the LB and UB mean occurrence estimations across those groups of the FoodEx at the second and third level are reported in Table 6.

In 404 samples the three individual stereoisomers were measured, in 797 samples the total HBCDD was analysed, while in 97 samples the three congeners and the total HBCDD were analysed simultaneously. For occurrence purposes estimations (Table 5 and 6), those 97 samples were included both for estimating the LB and UB mean for the sum of the individual stereoisomers as well as for the total HBCDD as measured. A more detailed analysis of the latter 97 samples was attempted out in order to identify possible relationships between the measured total HBCDD and the calculated sum of

the HBCDDs stereoisomers. Nevertheless, due to the high proportion of non-detects among those samples, it was not possible to identify any correlation.

'Fish offal' are the main contributors to the total level of HBCDDs across the food groups fish and other seafood at the second level of the FoodEx. In particular, the food group of 'Other fish offal' where only fish liver (cod and saithe liver) was reported, the contamination levels of total HBCDD are the highest among all food groups reaching up to 5.10 and 5.17 ng/g w.w. for LB and UB mean, respectively.

The α-HBCDD stereoisomer is the main contributor to the total level of HBCDDs in fish with the lower proportion of non-detects.

Overall in all food groups, the high proportion of non-detects has an impact on the estimation of the LB and UB, which in general leads to an increased difference between LB and UB. This effect is particularly emphasised when calculating the sum of the levels of the three individual stereoisomers. For this reason, particularly the UB might be overestimated because of the inclusion of non detected stereoisomers in the calculation of the UB sum.

In the case of the food group of 'Fish and other seafood' the availability of a statistically relevant number of analytical results of total HBCDD as measured confirms this observation. Therefore, the estimated mean LB and UB from the total HBCDD might be a more realistic estimation of HBCDDs contamination levels in fish.

Moreover, as reported in literature (see Chapter 5.2.1.1) higher HBCDD levels could be associated with higher fish fat content also in the present data base. Nevertheless, the relationship found between the percentage of fat and the HBCDD levels was biased by the high proportion of non-detects.

In Table D2 (Appendix D) the occurrence values of α-, β- and γ-HBCDD, the calculated sum of the individual stereoisomers and total HBCDD are reported for the food groups defined by Commission Regulation (EC) No. 1881/2006,[7] Annex section 5, on setting maximum levels for certain contaminants in foodstuffs. In addition, the food groups 'Other products' (ng/g w.w.) and 'Infant and baby food' (ng/g w.w.) were included to cover other food products that are relevant with respect to dietary exposure of adults and children.

Additionally, for illustrative purposes, in Table D3 (Appendix D) the highest percentiles (90^{th}, 95^{th}, 99^{th} percentiles) of occurrence are also reported across the food categories as defined by the above legislation on dioxins, furans and PCBs.

Table 6: Statistical description of mean concentrations of α-, β-, γ-HBCDD, the calculated sum of the individual stereoisomers and total HBCDD, across the second and third level of the FoodEx food classification system within the broad food group of 'Fish and other seafood'. HBCDD concentrations are reported on a wet weight basis (ng/g w.w.). The number of analytical results (n) and proportion of non-detects (ND, %) across HBCDDs compounds and food groups and mean fat content calculated from the original samples (%) is also reported.

				HBCDD compounds															Mean percentage of fat in the original sample (%)
				α-HBCDD			β-HBCDD			γ-HBCDD			HBCDD-sum (α+β+γ)			Total HBCDD			
Food groups (FoodEx_ Level 1)	Food groups (FoodEx_ Level 2)	Food groups (FoodEx_ Level 3)	TYPE	n	MEAN	ND (%)[a]	n	MEAN	ND (%)[a]	n	MEAN	ND (%)[a]	n	MEAN	ND (%)[a]	n	MEAN	ND (%)[a]	
Results expressed on a wet weight basis (ng/g w.w.)																			
Fish and other seafood			LB	501	0.58	71 %	501	0.02	90 %	501	0.03	87 %	501	0.63	.	894	0.98	61 %	6.42
			UB	501	1.03	71 %	501	0.54	90 %	501	0.55	87 %	501	2.11	.	894	1.16	61 %	6.42
	Fish meat		LB	377	0.42	80 %	377	0.01	92 %	377	0.04	89 %	377	0.47	.	630	0.48	63 %	8.15
			UB	377	0.94	80 %	377	0.54	92 %	377	0.55	89 %	377	2.02	.	630	0.68	63 %	8.15
	Fish products		LB	1	0.37	0 %	1	0	100 %	1	0.00	100 %	1	0.37	.	.	.	.	10.10
			UB	1	0.37	0 %	1	0.05	100 %	1	0.05	100 %	1	0.47	.	.	.	.	10.10
	Crustaceans		LB	26	0.05	73 %	26	0.02	88 %	26	<0.01	88 %	26	0.08	.	17	0.05	71 %	2.05
			UB	26	0.56	73 %	26	0.53	88 %	26	0.51	88 %	26	1.60	.	17	0.20	71 %	2.05
	Water molluscs		LB	57	0.41	46 %	57	0.07	72 %	57	0.06	65 %	57	0.53	.	132	0.01	95 %	1.51
			UB	57	0.59	46 %	57	0.26	72 %	57	0.25	65 %	57	1.09	.	132	0.20	95 %	1.51
	Fish offal		LB	40	2.65	15 %	40	0.00	100 %	40	0.00	100 %	40	2.65	.	115	3.57	33 %	11.75
			UB	40	2.80	15 %	40	1.00	100 %	40	1.00	100 %	40	4.80	.	115	3.69	33 %	11.75
		Fish roe	LB	.	.	.	.	.	.	.	.	.	.	.	.	4	1.57	0 %	11.75
			UB	.	.	.	.	.	.	.	.	.	.	.	.	4	1.57	0 %	11.75
		Other fish offal (fish liver)	LB	40	2.65	15 %	40	0	100 %	40	0	100 %	40	2.65	.	111	5.10	7 %	.
			UB	40	2.80	15 %	40	1	100 %	40	1	100 %	40	4.80	.	111	5.17	7 %	.

LB: lower bound; UB: upper bound.
(a): The column ND (%) indicates the percentage of results below the LOD or the LOQ.

5.2. Previously reported literature data on HBCDD occurrence

5.2.1. Occurrence in Food

5.2.1.1. Fish

HBCDDs are found in fish and seafood worldwide as they accumulate in both wild-caught species and farmed species. There is a substantial amount of data reported in the literature in fish worldwide, and therefore only a selection was included in the table focusing on wild caught fish and seafood from Europe as it was assumed that this would best reflect the fish and seafood related dietary exposure of the European population (Table B1, Appendix B). Data was included to cover Europe to a large degree (geographically), and to cover fish and seafood from various water bodies such as lakes and rivers, European seas like the Baltic Sea and the North Sea, and the Atlantic Ocean. Since some farmed fish is imported from outside Europe (e.g. salmon from Chile, tilapia from Ecuador and pangasius from Vietnam) (van Leeuwen et al., 2008) data on these imported species are also included. Data on commonly reported total-HBCDD were included, as well as data on the individual stereoisomers, and their sum.

Data reported in literature are presented in several ways, i.e. as determined by GC-MS (i.e. total-HBCDD), as individual stereoisomers (i.e. α-, β- and γ-HBCDD) and as the sum of these (i.e. total HBCDD). Of the stereoisomers, α-HBCDD predominates, followed by γ-HBCDD. B-HBCDD is occasionally detected, but mostly <LOD/LOQ. Literature data in some cases describes contamination-hotspots such as close to HBCDD production facilities (Allchin and Morris, 2003) and downstream industrialised areas (Eljarrat et al., 2004, 2005; Roosens et al., 2008; Sellström et al., 1998). Levels up to 10,275 ng/g w.w. were reported in fish from these locations. Although they do not reflect the general level of contamination, fish from these locations may be consumed, even on a regular basis. Levels in lean fish, e.g. cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), plaice (*Pleuronectes platessa*) and pike-perch (*Sander lucioperca*), are generally low (<1 ng/g w.w.). Higher levels are found in lipid rich fish such as barbel (*Barbus barbus*), eel (*Anguilla anguilla*), herring (*Clupea harengus*) and lake trout (*Salvelinus namaycush*) (see Table B1, Appendix B). In some cases, these higher levels are also associated with industrialised areas. Mussels, oysters and shrimps generally contained levels below 1 ng/g w.w., except for shrimps from the Western Scheldt (Janak et al., 2005) and mussels and oysters from Scotland coastal areas (Fernandes et al., 2008). Among the farmed species, levels of the individual stereoisomers were <0.1 ng/g w.w. in pangasius (Striped catfish, *Pangasianodon hypophthalmus*) and shrimps, and generally <1 ng/g w.w. in oily fish as eel and salmonides.

5.2.1.2. Food samples other than fish

The number of papers reporting on levels of HBCDDs in food samples other than fish in Europe is rather limited. The data reported are given as total HBCDD or as individual data for α-, β- and γ-HBCDD (Table 7). Due to the limited data published, some food data from the United States of America and Canada have been included for comparison.

Remberger et al. reported in 2004 analytical data for a number of environmental samples including non fish food samples. All food samples were collected in 1999 in Sweden.

Knutsen et al. (2008) performed an analysis of HBCDDs in fish, meat and dairy products in Norway. In Table 7 the occurrence data on food other than fish are reported. The highest values are found in eggs from gulls followed by chicken eggs.

The extent and the sources of contamination with PBDEs and HBCDD in home-produced eggs from free-foraging chicken of Belgian private owners were investigated by Covaci et al. (2009). Ten eggs

were collected in each period during autumn 2006 and spring 2007. Various factors, such as seasonal variability, exposure of chickens through diet (kitchen waste) and soil were assessed. PBDEs were more important than HBCDD in terms of concentration and detection frequency. Total HBCDD was found at median values of <0.4 ng/g fat in autumn and 2.9 ng/g fat in spring respectively. The highest total HBCDD value was 62 ng/g fat. The similarity between profiles and seasonal variations in the concentrations of HBCDDs in soil and eggs from free-ranging chicken indicate that soil is an important source, but not the only source.

In a small study, Päpke et al. (2010) reported on the results of HBCDDs in cow's milk, fish, mussel and lamb liver collected between 2008 and 2009. Values total HBCDD for 15 milk samples and 12 lamb liver samples were found below LOQ at 0.02 ng/g and 0.05 ng/g fresh weight, respectively. Total HBCDD in fish (n=1) and mussel (n=2) were found for total HBCDD at 0.6 ng/g fresh weight.

In 2010 Schechter et al. published results of a US market basket survey for a number of halogenated chemicals, including HBCDDs, determined in food samples. Ten samples of 31 distinct food types (310 samples total) were collected from five supermarkets on two separate occasions in Dallas, Texas (USA), in 2009. Total HBCDDs were present at the highest concentrations in canned sardines (0.593 ng/g w.w.), followed by fresh salmon (0.352 ng/g w.w.) and peanut butter (0.300 ng/g w.w.). Total HBCDD concentration was below the LOD in ground beef, canned tuna, cod, olive oil, canola oil, margarine, cereals, eggs, apples, potatoes, and dairy products. HBCDDs were detected in 13 out of 31 samples: in seven out of eight meats, in five out of seven fish, no eggs or dairy products, and in one out of seven vegetables.

Recent data from Canada show findings for HBCDDs in egg yolk samples (Rawn et al., 2009). At least one stereoisomer of HBCDD was detected in the majority (85 %) of the 162 egg yolks analysed from all regions of Canada. In general, α-HBCDD was detected the most frequently (83 %) and was the dominant contributor to the total HBCDD levels. Total HBCDD concentrations in the yolks ranged from below the LOD to 1,110 ng/g fat. HBCDDs were also analyzed in food supplements from the Canadian market containing fish oil and other types of oils (Rawn et al., 2010). Total HBCDD concentrations ranged from <0.003 ng/g to a maximum of 5.05 ng/g, which was observed in a salmon oil supplement. Oil made from seal contained total HBCDD at concentrations of 0.47 ng/g fat.

Ortiz et al. (2011) analysed 25 samples of fish oil samples used for feed and food. Total HBCDD ranged from 0.09 to 26.8 ng/g, with higher concentrations in fish oil intended for feed (average value of 9.69 ng/g) than in oil intended for food (average value of 1.14 ng/g). Concentrations of α-HBCDD (average value of 4.12 ng/g in feed samples and 0.48 ng/g in food samples) and γ-HBCDD (5.05 and 0.43 ng/g, respectively) were higher than those of β-HBCDD (0.52 and 0.19 ng/g, respectively) in most of the samples. Concentrations of HBCDDs were compared to concentrations of other pollutants and a correlation between dioxin and dioxin-like PCBs levels and HBCDDs levels was observed. Intake of HBCDDs was calculated for fish oil with human consumption purposes and ranged from 0.08 to 5.38 ng HBCDD/g, which could contribute significantly to total intake of HBCDDs. Enantiomeric fractions were also determined. No clear enrichment was observed for γ-HBCDD, while (-)-α-HBCDD enrichment was detected in some samples.

Studies analysing food samples other than fish for individual HBCDD stereoisomers are not available before 2007/2008. Analytical data for total HBCDD show highest values for samples of animal origin like beef, chicken, lamb and eggs, especially eggs from sea gulls. Since some years nearly all data reported are given on stereoisomer specific basis. Highest values in samples of animal origin are found for α-HBCDD. On the other hand, fish caught in rivers downstream of highly industrialised areas can show higher concentrations for γ-HBCDD (Eljarrat, 2010). This may be due to fresh contamination of γ-HBCDD, the dominating isomer of the technical product.

Table 7: Levels of HBCDDs in foodstuff (ng/g fat or ng/g wet weight) reported in the literature from different European countries.

Country	Year	Food type (N)	Fat content (%)	α-HBCDD	β-HBCDD	γ-HBCDD	Sum of HBCDD	Total HBCDD			Reference
								mean	median	range	
Sweden	1999	Lamb fat (n.r.)	87					1.4	n.r.	n.r.	Remberger et al., 2004[a]
		Pork fat (n.r.)	82					1.0	n.r.	n.r.	
		Beef fat (n.r.)	81					4.1	n.r.	n.r.	
		Veal fat (n.r.)	81					<1	n.r.	n.r.	
		Chicken (n.r.)	63					6.5	n.r.	n.r.	
		Egg yolk (n.r.)	25					9.4	n.r.	n.r.	
		Cow´s Milk (n.r.)	3.8					1.8	n.r.	n.r.	
Norway	2002-2006	Bovine meat (2)	17.8	LB 0.000 UB 0.020	LB 0.000 UB 0.020	LB 0.000 UB 0.020	n.r.				Knutsen et al., 2008[b]
		Sheep meat (1)	19.4	LB 0.000 UB 0.050	LB 0.000 UB 0.040	LB 0.000 UB 0.060	n.r.				
		Pork meat (1)	24.8	LB 0.021 UB 0.021	LB 0.000 UB 0.010	LB 0.007 UB 0.007	n.r.				
		Sausage (2)	16.4	LB 0.000 UB 0.010	LB 0.000 UB 0.010	LB 0.000 UB 0.010	n.r.				
		Liver pate (2)	21.1	LB 0.019 UB 0.019	LB 0.003 UB 0.011	LB 0.030 UB 0.041	n.r.				
		Dairy products (8)	52.1	LB 0.018 UB 0.223	LB 0.016 UB 0.161	LB 0.015 UB 0.268	n.r.				
		Hen eggs (6)	9.8	LB 0.095 UB 0.116	LB 0.011 UB 0.040	LB 0.008 UB 0.053	n.r.				
		Gull eggs (*Larus* spp.) (20)	9.4	LB 6.620 UB 6.620	n.r.	n.r.	n.r.				

Table 7: Continued.

Country	Year	Food type (N)	Fat content (%)	α-HBCDD	β-HBCDD	γ-HBCDD	Sum of HBCDD	Total HBCDD			Reference
								mean	media	range	
		Vegetable oil (2)	100	LB 0.000 UB 0.125	LB 0.000 UB 0.110	LB 0.000 UB 0.145					
		Ice cream (2)	10.3	LB 0.000 UB 0.010	LB 0.000 UB 0.010	LB 0.000 UB 0.000					
		Biscuits (7)	14	LB 0.009 UB 0.071	LB 0.008 UB 0.052	LB 0.015 UB 0.090					
		Banana (1)	0.1	LB 0.000 UB 0.010	LB 0.000 UB 0.010	LB 0.000 UB 0.020					
Northern Europe	2007-2009	Milk (15)	n.r.	n.r.	n.r.	n.r.	n.r.	<0.02	n.r.	<0.02	Päpke et al., 2010[(b)]
		Fish (1)	n.r.	0.48	0.09	0.08	n.r.	0.6	n.r.	0.6-0.6	
		Lamb liver (12)	n.r.	n.r.	n.r.	n.r.	n.r.	<0.05	n.r.	<0.05	
		Mussel (2)	n.r.	0.41	0.12	<0.06	n.r.	0.58	n.r.	0.15-1.01	

N: number of samples; n.r.: not reported.
(a): ng/g fat weight.
(b): ng/g wet weight.

5.2.1.3. Effects of processing

There are no data available on effects of processing on HBCDD levels in food. Due to the comparable chemical characteristics of HBCDDs with those of PBDEs and PBBs, a similar conclusion can be drawn for these latter compounds.

In general, HBCDDs are chemically stable lipophilic substances. Reduction of their content in processed foods may be mainly caused by loss of fat, rather than degradation. On the other hand, contact with BFR containing wrapping material may result in elevated contamination of the respective food due to migration.

5.2.2. Occurrence in human milk

Investigations on HBCDD levels in human milk are scarce. Most of the data are not stereoisomer-specific, but given as total HBCDD. Table 8 gives respective results in human samples from different European countries. For total HBCDD the concentrations ranged from 0.13 to 31 ng/g fat. Where reported, the mean and median levels were below 2 ng/g fat. In those samples that were analysed stereoisomer-specific (studies in France, Spain and UK), generally α-HBCDD predominates with levels between < 0.20 and 19.71 ng/g fat. Considerably higher levels were found in human milk in a study carried out in A Coruña in Spain, where the sum of the 3 HBCDD stereoisomers ranged from 3 to 188 ng/g fat with most samples showing higher levels for γ-HBCDD than for α-HBCDD. The reason for these high concentrations and the unusual stereoisomer pattern is not known. A few human milk samples from Barcelona analysed by the same authors showed significant lower levels (1.3-6.2 ng/g fat) which are in the same range as samples from other European countries that were analysed stereoisomer- specific (Eljarrat, personal communication, 2011).

Fängström et al. (2008) analysed human milk pools from Sweden which were archived between 1980 and 2004. The Stockholm human milk pools showed a seven times increase of HBCDD concentrations between 1980 and 2002 with somewhat decreasing levels between 2002 and the end of the study in 2003/2004 (see Figure 8). While the pool from 1980 had a HBCDD concentration of around 0.08 ng/g fat, the respective pool from 2004 had a concentration of 0.39 ng/g fat. Pooled human milk samples from Stockholm collected in 2009 and 2010 showed somewhat higher levels at 0.62 and 0.80 ng/g fat.

Eljarrat et al. (2009) analysed the enantiomeric pattern of human milk samples from Spain. Their results show a selective enantiomeric enrichment in the human body. As regards α-HBCDD, an enrichment of the (-)-enantiomer was observed. However, in the case of γ-HBCDD, no clear preference for one or the other enantiomer was found.

The enantiomer fractions (EFs) for the 3 major HBCDD stereoisomers profiles in human milk were also determined by Abdallah and Harrad (2011). While the EFs of β-HBCDD (average 0.49) and γ-HBCDD (average 0.51) showed no significant deviations from racemic, substantial enrichment of the (−)-α-HBCDD enantiomer was evident from the EFs of this diastereomer (average 0.29).

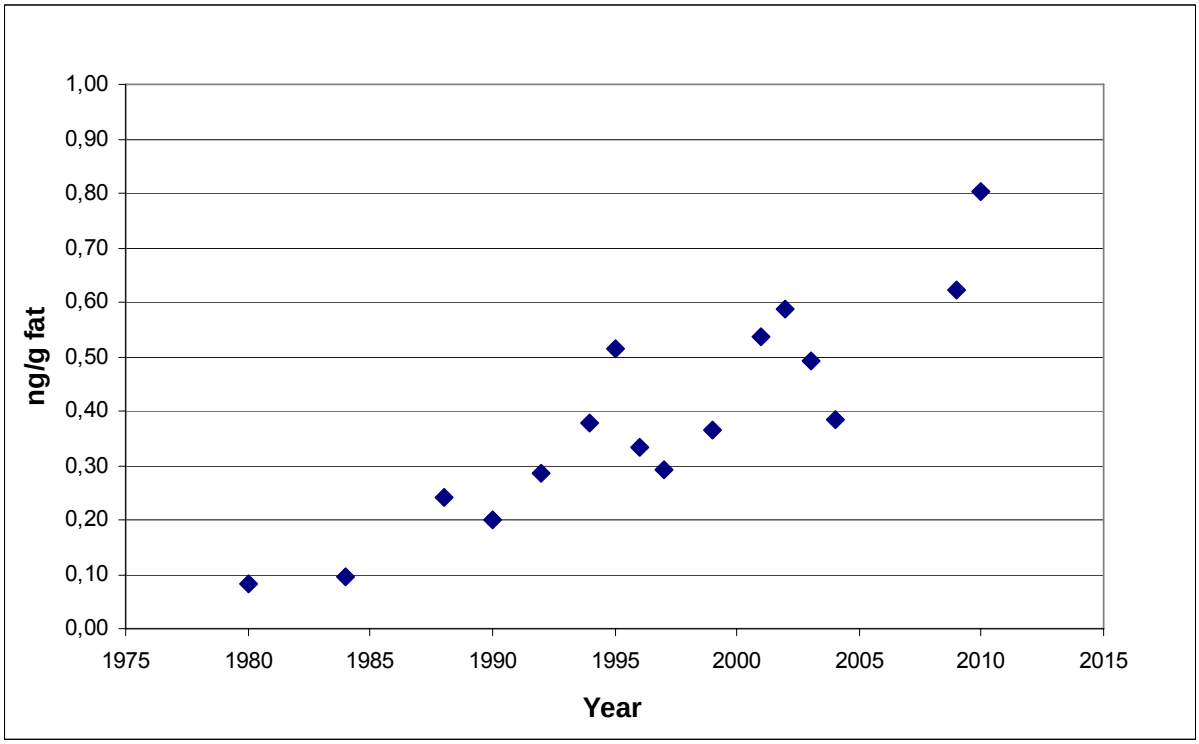

Figure 8: Temporal trend for total HBCDD concentrations in human milk from Stockholm between 1980 and 2010 (Fangström et al., 2008 (data from 1980-2008); Bergman et al., 2010 (data from 2009-2010)).

Table 8: Levels of HBCDDs in human milk (ng/g fat) from different countries.

Country	Year	N	α-HBCDD	β-HBCDD	γ-HBCDD	Sum of HBCDD	Total HBCDD mean	Total HBCDD median	Total HBCDD range	Reference
France	2005	23	2.5-5 [(a)]	n.r.	n.r	n.r.				Antignac et al., 2008
Belgium	2006	1[(h)]	1.5	<0.8	<0.8	n.r.				Colles et al., 2008
Belgium	2006	22[(g)]					n.r.	n.r.	<2.1 – 5.7	Roosens et al., 2010a
Spain	2006-2007	33[(b)]	<LOD-122	< LOD	<LOD-176	3-188 Mean: 47 Median: 27	47	27	3-188	Eljarrat et al., 2009
Spain	2009	6	<0.2-3.6	<1.0-<3.3	<0.3-6.2	1.3-6.2				Eljarrat, personal communication, 2011
Sweden	1996-2006	177[(c)]					0.39	0.25	<0.20-7.8	Lignell et al., 2008
Norway	2000-2002	1[(d)]					n.r.	n.r.	0.13	Polder et al., 2008
Norway	2003-2009	310[(e)]					1.7	0.86	<0.20-31	Thomsen et al., 2010
Russia (Murmansk)	2000	14[(i)]					0.47	0.45	0.20-1.15	Polder et al., 2008
Russia (Arkhangelsk)	2002	23[(j)]					0.71	0.62	0.24-1.67	Polder et al., 2008
UK	n.r.	34	3.17[(f)] (0.75-19.71)	0.30 (0.08-0.75)	0.56 (0.13-2.29)	3.83 (1.04-22.37)				Abdallah and Harrad, 2011

N: number of samples; n.r.: not reported.
(a): 7 samples > LOD.
(b): 30 samples > LOQ. The analysis was performed stereoisomer-specific and the sum of the 3 stereoisomers is reported as total HBCDD in the publication.
(c): 66 % of samples < LOQ, Total levels based on medium bound.
(d): 1 of 10 samples above LOQ.
(e): 56.8 % of samples > LOQ.
(f): Median.
(g): Pools.
(h): Pool (178 samples).
(i): 8 of 14 samples above LOQ.
(j): 3 of 23 samples above LOQ.

6. Food consumption

6.1. EFSA's Comprehensive European Food Consumption Database

In most of the latest EFSA opinions concerning contaminants, the EFSA Concise European Food Consumption Database (Concise Database) was used in order to assess dietary exposure. The Concise Database was operational since 2008 and contained information from individual dietary surveys from the majority of EU Member States. However, it was intended to be used as a screening tool for dietary exposure assessment as well as a first step towards generating a more comprehensive database.

As a next step, EFSA established in 2010 the Comprehensive European Food Consumption Database (Comprehensive Database). This is built on existing information for adults and children at a detailed level. Through a procurement project (DPPA/EFSA/DATEX/2008) 22 different Member States provided food consumption data at the individual level to EFSA collected within the most recent national dietary surveys. Detailed information on the 32 dietary surveys included in the Comprehensive Database can be found in the recently published guidance on the 'Use of the EFSA Comprehensive European Food Consumption Database in exposure assessment' (EFSA, 2011a). All food consumption data were codified according to the FoodEx classification system which has been developed by the EFSA DCM Unit in 2009.

According to the guidance document (EFSA, 2011a), the different age classes applied for classifying the subjects from the different dietary studies are:

- Infants: up to and including 11 months
- Toddlers: from 12 up to and including 35 months of age
- Other children: from 36 months up to and including 9 years of age
- Adolescents: from 10 up to and including 17 years of age
- Adults: from 18 up to and including 64 years of age
- Elderly: from 65 up to and including 74 years of age
- Very elderly: from 75 years of age and older

Despite the improved data quality and quantity, still methodological differences exist between the various country surveys included in the Comprehensive Database, making these data not fully suitable for country-to-country comparisons.

On the other hand, the food consumption data compiled in the Comprehensive Database are the most complete and detailed data currently available in the EU. Due to the detailed structure of the food classification (FoodEx) the Comprehensive Database will allow the estimation of the intake for detailed food groups.

6.2. Food consumption data for specific age and consumers group

6.2.1. Infants and young children

Estimating the potential HBCDDs dietary exposure for infants from human milk and infant formula requires information about the quantity of liquid consumed per day and the duration over which such consumption occurs. According to the Institute of Medicine of the U.S. National Academies of Sciences (IOM), average human milk consumption is about 750-800 g per day (range, 450-1,200 g per day) for the first 4-5 months of life. Infant birth weight and nursing frequency have been shown to influence consumption (IOM, 1991). The WHO related human milk consumption to body weight rather than to age, with an estimated 125 mL/kg or 763 mL for a 3 month old child weighing 6.1 kg (Onyango et al., 2002). According to the German DONALD study, mean consumption of infant formula for a three month old child weighing on average 6.1 kg, was 780 mL per day, with a 95th percentile consumption of 1,060 mL/day (Kersting et al., 1998). A common mean of 800 mL per

day will be used in this opinion for consumption of human milk and infant formula when calculating exposure, with a high of 1,200 mL per day.

6.2.2. Children

The Comprehensive Database contains detailed food consumption data for children. In particular, results from consumption surveys from 13 different Member States for children gathered by means of the EFSA Article 36 project 'Individual food consumption data and exposure assessment studies for children' (acronym EXPOCHI) (Huybrechts et al., in press) were incorporated in the database. Consumption records were codified according to the FoodEx classification system which has been developed by the DCM Unit in 2009. The consumption data for children from the Comprehensive Database (including the EXPOCHI data) are used in this opinion for the estimation of HBCDDs dietary intake of children according to the different age groups as described in Chapter 6.1.

6.2.3. High and frequent fish consumers

Due to the comparatively elevated HBCDDs levels of fish and seafood products, a specific diet characterised by high fish consumption might lead to higher dietary intake for this population, people who might consume fish every day (with particular focus on 'Fish meat' only, regardless of the fish species; FoodEx level 2) like fishermen or fishmongers might be at even higher risk. In order to estimate the dietary intake of HBCDDs for this specific scenario, a daily consumption per body weight of 2.6 g/kg b.w. of fish meat eaten by the European population was retrieved from the Comprehensive Database.[16] This value was identified as the 95th percentile of consumers only, by selecting the dietary surveys with more than one day dietary record, where more than 60 individuals participated in the survey.

Additionally, another specific group of the population that might be exposed to high levels of HBCDDs are consumers of fish liver. Fish liver is a food product rarely consumed in Europe. Nevertheless, due to the high levels of HBCDDs in this food product, even average consumers might be highly exposed. Among those surveys with more than one day dietary records from the Comprehensive Database, only 14 subjects declared having consumed 'Other fish offal' (FoodEx level 3, where the food consumed was specifically described as fish liver) once within the survey period, across four age classes and four consumption surveys. Due to the limited consumption information available, an average portion size of fish liver was estimated from the survey where at least more than one subject was present. The average portion size of 83.25 g per person was finally estimated from eight participants (adults and elderly) of the French dietary survey (AFSSA, 2009; Lioret et al., 2010; Dubuisson et al., 2010) having consumed cod liver once during the survey period (7 days), which corresponds to 0.18 g/kg b.w. per day (11.9 g per person per day).

Furthermore, in a Norwegian food frequency questionnaire carried out in 2000 on 5,502 individuals from inland and costal areas (Bergsten, 2004), consumption of cod and saithe liver was investigated. Daily food consumption of fish liver was estimated on the basis of the frequencies of reporting and on the portion sizes. In the case of medium-sized portions, the highest mean consumption is reported for men living in the costal area with up to 1.1 g of fish liver per person per day, whereas for 95th percentile consumers the daily portion reaches up to 3.7 g for men and 2.7 g for women. When considering a large portion size of 65 and 55 g for men and women, respectively, the estimated fish liver consumption for high consumers was not higher than 7 g per person per day, which corresponds to 0.12 g/kg b.w. per day (assuming a body weight of 60 kg). Therefore, aiming at a conservative approach, an estimated daily consumption of 0.18 g/kg b.w. per day extrapolated from the Comprehensive Database will be used for assessing the exposure of fish liver consumers. Additionally, considering the consumption frequencies in the Norwegian population as collected using

[16] See food consumption statistics according to the FoodEx food classification system for the total population and for consumers of respective food groups only, at http://www.efsa.europa.eu/en/datex/datexfooddb.htm.

the food frequency questionnaire, a medium-sized portion of 35 and 30 g of fish liver (cod or saithe liver), respectively for men and women was consumed 'once a week or more' by up to 0.6 % of the population, '1-3 times a year' by up to 4.9 %, 'a few times a year' by up to 31 %, and up to 89 % of the population declared that they never consumed fish liver of cod or saithe origin. Taking into account this available information, a possible scenario of consumers of about 83 g of fish liver once a week (corresponding to 0.18 g/kg b.w. day), was considered.

6.2.4. Consumers of food supplements

Another group of people that might have an elevated HBCDDs intake are consumers of food supplements, especially if these consist of fish oil capsules or fish liver oil. Since the consumption recommended on the package varies according to the different forms and brands (e.g. one tea spoon or one table spoon, from one to four capsules per day), the CONTAM Panel assumed a maximum daily consumption of 15 mL of fish oil supplements in its liquid form, and 4 g of fish oil as capsules or tablets for the exposure estimate to cover a worst case scenario.

7. Human dietary exposure assessment

For this opinion the mean and the 95th percentile of chronic dietary HBCDDs exposure (ng/kg b.w. per day) by food group were calculated separately for each European country for the whole population using consumption data recorded at the individual level from the Comprehensive Database. Due to the methodological differences among the consumption surveys included in the Comprehensive Database, chronic dietary HBCDDs exposure was estimated separately by consumption surveys.

Following the recently published guidance on the use of the Comprehensive Database for exposure assessment (EFSA, 2011a), dietary surveys with only one day record per subject were not considered for the calculation of chronic dietary exposure, as they are not adequate to assess repeated exposure. In the remaining dietary surveys, one day records were also present for some of the subjects, despite the study protocol prescribed more reporting days per individual. In line with the guidance document (EFSA, 2011a) these subjects were excluded from the calculation of chronic dietary exposure. For the present assessment, food consumption data were available from 28 different dietary surveys carried out in 17 different European countries as follows:

- Infants: 2 European countries: 2 dietary surveys;
- Toddlers: 7 European countries: 9 dietary surveys;
- Other children: 13 European countries; 17 dietary surveys;
- Adolescents: 10 European countries; 12 dietary surveys;
- Adults: 14 European countries; 15 dietary surveys;
- Elderly: 7 European countries; 7 dietary surveys;
- Very elderly: 6 European countries; 6 dietary surveys.

The limited occurrence data available in the food group 'Food for infants and small children' (only 16 samples) were not considered adequate to represent products consumed by infants below one year of age (e.g. only three samples described as infant formula and follow-up formula) and toddlers (e.g. only 13 samples described as ready to eat meal for infants and young children). Considering also the restricted number of consumption data for infants (only two dietary surveys available), it was decided not appropriate to assess the dietary exposure to HBCDDs for infants and toddlers.

The dietary surveys considered for the chronic dietary exposure assessment and number of subjects in the different age classes are presented in Table C1 (Appendix C).

7.1. Linkage of occurrence and consumption data

A detailed analysis of the occurrence levels of α-, β-, γ-HBCDD, the sum of the three stereoisomers and total HBCDD (where available) was performed on the basis of the data submitted for 6 broad food groups ('Eggs and egg products', 'Milk and dairy products', 'Meat and meat products', 'Fish and other seafood', 'Products for special nutritional use' and 'Food for infants and small children'), as explained in Chapter 5.1.5. This occurrence data were combined with the food consumption data in order to assess dietary exposure.

With the aim of considering all possible sources of dietary exposure, assumptions have been made for those food groups suspected to be contaminated by HBCDDs but for which no or insufficient information was provided by European countries. In particular when data providers were reporting their consumption into the Comprehensive Database, the use of food description like 'Composite food (including frozen products)' from the FoodEx was discouraged and suggested to be used only if no other possibilities were available. Most countries managed to split most composite foods into their respective ingredients with the exception of Latvia, Sweden and Slovakia (10 %, 8 % and 7 % of food records classified under 'Composite foods', respectively) (EFSA, 2011b). When the main ingredient of a composite food derives from a food group which is suspected to contain HBCDDs, the LB and UB mean occurrence of the composite food has been associated with the ones of the broad food group for which occurrence data is available. For example composite foods categorised as 'Egg-based meal' would mainly contain eggs and consequently these have been associate with LB and UB mean occurrence of the broad food group 'Eggs and eggs products'.

Additional data analysis has been carried out taking into account the proportion of non-detects. As shown in Table 5 and 6, the proportion of non-detects ranged from 15 to 100 % across the individual HBCDD stereoisomers and from 33 % to 100 % for total HBCDD. Notwithstanding the variable proportion of non-detects among the different stereoisomers in the same sample, an estimation of the LB and UB mean of the sum of the three stereoisomers (HBCDD-sum (α+β+γ)) was performed. As a consequence, especially where all the three stereoisomers were non-detects, the estimation of LB and UB mean of the sum (HBCDD-sum (α+β+γ)) was mainly driven by the reported LODs or LOQs. The estimated occurrence values in those cases were more reflecting the variation of LOD and LOQ values and the performance of the analytical methods rather than the actual HBCDDs levels.

In the food group 'Fish and other seafood' in 894 samples the total HBCDD was measured while for 501 samples the sum of the three stereoisomers was calculated. Due to the high proportion of non-detects for β- and γ-HBCDD, the sum might be biased by the LODs and LOQs reported for these stereoisomers. Therefore, the mean LB and UB for total HBCDD is considered more accurate than the calculated sum of the three individual stereoisomers, and therefore more suitable for dietary exposure assessment purposes. Only in the case of the food group 'Fish and other seafood' this choice could be made because for the remaining food groups for relatively few samples total HBCDD was measured, and these could not be considered as representative for the entire group.

Following these considerations, the CONTAM Panel decided to base the dietary exposure to HBCDDs for the food groups of 'Eggs and egg products', 'Milk and dairy products', 'Meat and meat products' on the mean occurrence data estimated for the sum of the individual HBCDDs stereoisomers individual level (α-, β- and γ-HBCCD), whereas for 'Fish and other seafood' the mean level for total HBCDD was applied.

Table D1 (Appendix D) describes LB and UB mean occurrence values used for the dietary exposure assessment calculation, including ad-hoc food groups and assumptions as mentioned above.

HBCDDs levels in 'Products for special nutritional use' as in Table 4 are applied in the dietary exposure estimation of specific population groups, as described in Chapter 7.4.

7.2. Current estimates of mean and high dietary exposure to HBCDDs for the general population

Following the considerations on the available occurrence and consumption data, total dietary exposure to HBCDDs has been assessed for the general population, for average and 95th percentile consumers. Estimation of dietary exposure to HBCDDs for infants and toddlers was considered not appropriate due to the limited occurrence data available in the food group 'Food for infants and small children' (only 16 samples). In Table 9 and 10 the chronic total dietary intake of respectively average and 95th percentile consumers to HBCDDs are reported per European dietary survey. Summary statistics (minimum, median and maximum estimates) across dietary surveys in European countries are also provided and have been estimated taking into account the recommendations for chronic dietary exposure mentioned in the guidance document 'Guidance on the use of Comprehensive European Food Consumption Database in exposure assessment' (EFSA, 2011a).

The mean dietary exposure to HBCDDs across dietary surveys in European countries was estimated for children from three to ten years old ('Other children') and is between 0.15 to 1.85 ng/kg b.w. per day (minimum LB and maximum UB, respectively). Total dietary exposure for adults is about half the exposure for 'Other children', with minimum LB and maximum UB of respectively 0.09 and 0.99 ng/kg b.w. per day. Exposure to HBCDDs is decreasing with increasing age down to 0.06 and 0.54 ng/kg b.w. per day (minimum LB and maximum UB, respectively) for 'Very elderly'.

Similar exposure patterns across age classes are found for dietary intake of high consumers (95th percentile). The minimum LB and maximum UB dietary intake of HBCDDs across European countries and dietary surveys are between 0.80 and 4.46 ng/kg b.w. per day for 'Other children', followed by 'Adults' with 0.39 and 2.07 ng/kg b.w. per day, and down to 'Very elderly' with 0.27 and 1.26 ng/kg b.w. per day.

The variation observed in dietary exposure between countries and surveys is only influenced by different consumption patterns, since the HBCDD concentrations in the selected food groups of the FoodEx food classification system were calculated at European level.

Table 9: Mean chronic dietary exposure (ng/kg b.w. per day) to HBCDDs for total population across European dietary surveys. The total dietary intake was estimated using the lower bound (LB) and upper bound (UB) HBCDD concentrations from the calculated sum of α-, β- and γ-HBCCD for all selected food groups, except for the food group of 'Fish and seafood' where data on total HBCDD were available. Total dietary intake estimates are reported by age classes and European dietary surveys. In addition, statistical descriptors like minimum, median and maximum per age classes are given.

Dietary survey[(a)]	Other children		Adolescents		Adults		Elderly		Very elderly	
	LB	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**	**LB**	**UB**
BE/1			0.15	0.37	0.18	0.39	0.18	0.37	0.17	0.36
BE/2	0.39	1.46								
BG	0.28	1.05								
CY			0.16	0.45						
CZ	0.32	0.97	0.25	0.65	0.14	0.35				
DK	0.34	1.27	0.17	0.64	0.14	0.43	0.18	0.47	0.22	0.54
FI/1	0.36	1.75								
FI/2					0.19	0.52	0.25	0.58		
FI/3	0.51	1.41								
FR	0.67	1.48	0.29	0.64	0.25	0.50	0.27	0.52	0.26	0.51
DE/1	0.35	0.92								
DE/2	0.36	0.98								
DE/3	0.38	0.97								
DE/4			0.09	0.26	0.14	0.32	0.17	0.34	0.17	0.35
GR	0.36	1.17								
HU					0.09	0.33	0.06	0.27	0.06	0.28
IE					0.16	0.44				
IT	0.82	1.66	0.36	0.78	0.26	0.55	0.26	0.53	0.20	0.45
LT	0.15	0.47	0.09	0.30	0.15	0.32				
NL/1					0.09	0.36				
NL/2	0.26	1.15								
ES/1					0.40	0.83				
ES/2			0.30	0.78	0.48	0.99				
ES/3	0.76	1.85	0.43	1.01						
ES/4	0.60	1.70	0.48	1.06						
SE/1					0.32	0.63				
SE/2	0.61	1.52	0.36	0.90						
UK					0.19	0.43				
Minimum	**0.15**	**0.47**	**0.09**	**0.26**	**0.09**	**0.32**	**0.06**	**0.27**	**0.06**	**0.28**
Median	**0.36**	**1.27**	**0.27**	**0.64**	**0.18**	**0.43**	**0.18**	**0.47**	**0.18**	**0.40**
Maximum	**0.82**	**1.85**	**0.48**	**1.06**	**0.48**	**0.99**	**0.27**	**0.58**	**0.26**	**0.54**

(a): Original acronyms of the dietary surveys and the number of subjects are given in Table C1 (Appendix C).

Table 10: High (95th percentile) chronic dietary exposure (ng/kg b.w. per day) to HBCDDs for the general population across European dietary surveys. The total dietary intake was estimated using the lower bound (LB) and upper bound (UB) HBCDD concentrations from the calculated sum of α-, β- and γ-HBCDD for all selected food groups, except for the food group of 'Fish and other seafood' where data on total HBCDD were available. Total dietary intake estimates are reported by age classes and European dietary surveys. In addition, statistical descriptors like minimum, median and maximum per age classes are given.

Dietary survey[a]	Other children		Adolescents		Adults		Elderly		Very elderly	
	LB	UB	LB	UB	LB	UB	LB	UB	LB	UB
BE/1			0.69	1.17	0.82	1.29	0.73	1.13	0.80	1.26
BE/2	1.70	3.34								
BG	1.66	2.83								
CY			0.66	1.27						
CZ	1.60	2.83	1.34	2.34	0.76	1.23				
DK	1.00	2.42	0.47	1.36	0.39	0.88	0.42	0.93	0.60[b]	1.12[b]
FI/1	1.23	3.26								
FI/2					0.76	1.32	0.92	1.51		
FI/3	2.54	3.83								
FR	1.82	3.07	0.84	1.47	0.66	1.08	0.65	1.03	0.60	1.03
DE/1	1.71	2.44								
DE/2	1.65	2.63								
DE/3	1.66	2.66								
DE/4			0.62	0.95	0.75	1.17	0.82	1.27	0.84	1.24
GR	1.52	2.87								
HU					0.46	0.84	0.32	0.63	0.27	0.56
IE					0.50	0.96				
IT	2.71	4.46	1.14	1.90	0.80	1.34	0.86	1.37	0.67	1.13
LT	0.80	1.30	0.54	0.86	0.71	1.15				
NL/1					0.53	0.96				
NL/2	1.51	2.74								
ES/1					1.25	2.03				
ES/2			0.87	1.67	1.22	2.07				
ES/3	2.40	4.06	1.42	2.38						
ES/4	2.14	3.93	1.66	2.68						
SE/1					0.90	1.38				
SE/2	2.05	3.48	1.39	2.22						
UK					0.55	0.95				
Minimum	**0.80**	**1.30**	**0.47**	**0.86**	**0.39**	**0.84**	**0.32**	**0.63**	**0.27**	**0.56**
Median	**1.66**	**2.87**	**0.86**	**1.57**	**0.75**	**1.17**	**0.73**	**1.13**	**0.67**	**1.13**
Maximum	**2.71**	**4.46**	**1.66**	**2.68**	**1.25**	**2.07**	**0.92**	**1.51**	**0.84**	**1.26**

(a): Original acronyms of the dietary surveys and the number of subjects are given in Table C1 (Appendix C);
(b): P95 estimates for dietary surveys/age classes with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they should not be considered in the risk characterisation.

7.3. Relative contributions of different food groups to the HBCDDs dietary exposure

In order to provide an overview of the range of relative contribution of each of the selected six food groups to the overall HBCDDs dietary intake for each age class, the minimum, median and maximum LB and UB intake for each food group was considered across European dietary surveys and reported in Table 11. Dietary intake reflects the pattern of consumption figures of each age class and the respective country as well as occurrence values.

Table 11: Relative contribution (%) to the overall dietary intake of HBCDDs of the six selected food groups across different age classes of the general population. The median and the range (minimum and maximum) lower (LB) and upper bound (UB) contributions of each food groups to the overall diet across the European dietary surveys are given. Percentages refer to the intake of HBCDDs estimated from LB and UB mean values occurrence from the calculated sum of α-, β- and γ-HBCDD for all selected food groups, except for the food groups of fish and seafood where total HBCDD was available.

	Age classes									
	Other children		Adolescents		Adults		Elderly		Very elderly	
	Median	(Min-Max)	Median	(Min-Max)	Median	(Min-Max)	Median	(Min-Max)	Median	(Min-Max)
Food groups										
					LB (%)					
Eggs and egg products	2.1	(0.5 - 4.4)	2.0	(0.5 - 5.4)	1.7	(1 - 6)	1.3	(1 - 7.3)	1.4	(1.1 - 7.1)
Fish meat and products	83.2	(55.6 - 91.3)	83.0	(60.6 - 88.7)	84.4	(63.9 - 90.6)	88.2	(55.1 - 91)	87.8	(55 - 91)
Fish offal	0.0	(0 - 23.9)	0.2	(0 - 15.4)	0.4	(0 - 10.6)	0.1	(0 - 7.4)	0.5	(0 - 8.7)
Meat and meat products	6.8	(4.3 - 17.6)	8.9	(5.9 - 20.6)	8.3	(4.4 - 24.5)	6.6	(4.7 - 29.1)	6.1	(5.2 - 29.7)
Milk and dairy products	5.1	(1.9 - 12.7)	3.4	(1.8 - 8.6)	2.3	(1.5 - 7.5)	2.0	(1.3 - 8.5)	2.2	(1.6 - 8.2)
Water molluscs and Crustaceans	0.2	(0 - 1.1)	0.5	(0 - 2.4)	1.0	(0 - 2)	0.6	(0 - 1.2)	0.7	(0 - 1.1)
					UB (%) [(a)]					
Eggs and egg products	2.8	(0.7 - 5.2)	2.7	(0.7 - 6.2)	2.6	(1.8 - 6.2)	2.6	(1.8 - 6.2)	2.5	(1.9 - 6.1)
Fish meat and products	39.4	(21.3 - 59.4)	43.0	(22.1 - 54.9)	51.3	(24 - 61.5)	59.4	(17.4 - 64.1)	56.3	(17.6 - 63.3)
Fish offal	0.0	(0 - 6.5)	0.1	(0 - 4)	0.2	(0 - 5.3)	0.1	(0 - 2.9)	0.3	(0 - 3.5)
Meat and meat products	9.8	(8.4 - 21.1)	13.5	(10.8 - 26.2)	15.3	(9 - 28.4)	12.7	(8.3 - 27.2)	11.6	(10.6 - 28.1)
Milk and dairy products	46.0	(24.1 - 67.9)	34.1	(21.2 - 57.6)	28.7	(18.2 - 51.4)	24.4	(17.4 - 49.2)	25.8	(20.6 - 48.2)
Water molluscs and Crustaceans	0.2	(0 - 4.9)	1.7	(0 - 9.6)	1.4	(0 - 7.6)	1.1	(0 - 5.4)	1.6	(0 - 3.8)

(a): Due to the high proportion on non-detects across the individual stereoisomers, the calculation of the UB sum might be overestimated. A particular high impact on the UB calculation can be observed in the case of 'Milk and dairy products', leading to a possible overestimation of the UB contribution to the overall HBCDD intake of this food group.

As described in Table D1 (Appendix D), the food group of 'Fish and other seafood' (FoodEx level 1) has been disaggregated into three ad-hoc food groups defined as 'Fish meat and products', 'Fish offal' and 'Water molluscs and crustaceans'. These ad-hoc food groups were created in order to merge FoodEx food groups at the most disaggregated level (FoodEx level 4, as described in Table 5) with similar occurrence figures and similar consumption patterns. This approach enables the evaluation of the separate contribution of 'Fish meat and products', 'Fish offal' and 'Water molluscs and crustaceans' to the overall HBCDDs dietary intake.

Considering the LB estimates, the contribution of 'Fish meat and products' to the median intake of HBCDDs across European dietary surveys varies from 83 to 88.2 % for the different age classes. In the case of 'Water molluscs and crustaceans' the contribution is about 1 %, whereas for 'Fish offal' this is 0.5 %. A considerable lower contribution to the dietary intake of HBCDDs is observed when the UB estimates are considered. For 'Fish meat and products' the contribution is 39.4 to 59.4 % across all age classes. Overall, the food groups 'Fish meat and products', 'Fish offal', and 'Water molluscs and crustaceans' together represent the main source of HBCDDs intake in the overall diet.

The second highest dietary source of dietary exposure to HBCDDs is the food group 'Meat and meat products', with median LB contribution across European dietary surveys ranging from 6.1 to 8.9 % and median UB from 9.8 to 15.3 % for different age classes.

The food group 'Eggs and egg products' contributes 1.3 to 2.1 % to the overall HBCDDs intake for the different age classes when considering the median LB across European dietary surveys, considering the median UB the contribution varies from 2.5 to 2.8 % for the different age classes.

For the food group 'Milk and dairy products', the median LB contribution ranges from 2 to 5.1 % for different age classes, whereas considering the median UB the contribution ranges from 24.4 to 46 % within the different age classes.

As previously mentioned, the high proportion of non-detects across the individual stereoisomers may have lead to overestimation of the upper bound sum. A particular high impact on the UB calculation can be observed in the case of 'Milk and dairy products' where the proportion of non-detects reported in 74 samples reached up to 74 to 96 % across stereoisomers. Therefore, the UB contribution of 'Milk and dairy products' to the overall HBCDDs intake can be considered to be an overestimation.

7.4. Dietary exposure to specific sub-groups of the population

7.4.1. Breast-fed infants (less that 1 year old)

For the exposure assessment of breast-fed infants an age of three months was selected, equivalent to a weight of about 6.1 kg, with an estimated average daily consumption of about 800 mL and a high consumption of 1,200 mL of human milk, each with a mean fat content of 3.5 %.

The exposure scenario based on average human milk consumption and the reported range for α-HBCDD in human milk (see Table 8) results in daily exposures of 0.90-90.5 ng/kg b.w. For infants with high human milk consumption the respective daily exposures range from 1.4-136 ng/kg b.w. This estimation does not include the occurrence data from the study carried out in A Coruña (Eljarrat et al., 2009) because of the unusual isomer pattern (γ-HBCDD more predominant than α-HBCDD) and the extremely high levels, which may point to a specific contamination source.

The exposure scenario based on average human milk consumption and the reported range for total-HBCDDs in human milk results in daily exposures of 0.60-142 ng/kg b.w. For infants with high human milk consumption the respective daily exposures range from 0.90-213 ng/kg b.w.

7.4.2. People following specific diets

7.4.2.1. High and frequent fish consumers

High consumption of fish is considered as a special diet with specific concern for dietary exposure to HBCDDs.

Furthermore, among the high fish consumers, people who might consume fish every day (with particular focus on 'Fish meat' consumption), e.g. fishermen or fishmongers, might be at even higher risk. In the case of these frequent and high consumers of fish meat, daily fish consumption (2.6 ng/kg b.w. per day) was retrieved from the Comprehensive Database, as described in Chapter 6.2, and combined with the total HBCDD occurrence values of 'Fish meat' (as described in Table 6). The intake estimations of total HBCDD for high and frequent fish consumers are reported in Table 12.

Table 12: Dietary exposure to total HBCDD as measured due to high and frequent consumption of 'Fish meat' to the basic diet. Number of analytical results (N), and lower bound (LB) and upper bound (UB) occurrence means used for the intake estimation are also reported.

	Mean concentration (ng/g w.w.)		**Additional intake of HBCDDs (assuming 2.6 g/kg b.w. per day fish meat consumption) (ng/kg b.w. per day)**		**Intake of HBCDDs from the basic diet (min LB and max UB)[(a)] (ng/kg b.w. per day)**		**Total intake of HBCDDs for high and frequent fish consumers (ng/kg b.w. per day)**	
	LB	UB	LB	UB	LB	UB	LB	UB
Total HBCDD	0.48	0.68	1.25	1.77	0.09	0.99	1.34	2.76

(a): Minimum LB and maximum UB intake estimates for adults across European countries (Table 9).

7.4.2.2. Consumption of fish liver

Beside the high consumption of fish, a special diet with specific concern of HBCDDs exposure could be the frequent consumption of fish liver. Fish liver is a traditional food especially in the coastal population of the Northern European countries. Although rarely consumed overall Europe, it might represent for certain regions a source of dietary exposure to HBCDDs and particularly to α-HBCDD.

In the case of frequent fish liver consumers, an average fish liver portion (83 g) was deduced from the information in the Comprehensive Database, and combined with the occurrence data for HBCDDs of 'Other fish offal' (FoodEx level 3) only including fish liver products (see Table 6). On the basis of the results of a Norwegian food frequency questionnaire, simulation of consumption frequency of once every week was carried out. Translating the frequency into daily consumption an average consumption of fish liver of 0.18 g/kg b.w. day was assumed, as described in Chapter 6.2. The intake estimations of HBCDDs for fish liver consumers are reported in Table 13.

Table 13: Dietary exposure (upper bound (UB) and lower bound (LB)) to the total HBCDD as measured due to frequent consumption of fish liver to the basic diet and simulation of frequency of consumption once per week. Number of analytical results (N), and lower and upper bound occurrence means (LB and UB) used for the intake estimation are also reported.

	Mean concentration (ng/g w.w.)		Additional intake of HBCDDs (ng/kg b.w. day)		Intake of HBCDDs from the basic diet (min LB and max UB)[(a)] (ng/kg b.w. per day)		Total intake of HBCDDs for fish liver consumers	
	LB	UB	LB	UB	LB	UB	LB	UB
Total HBCDD	5.10	5.17	0.94	0.95	0.09	0.99	1.03	1.94

(a): Minimum LB and maximum UB intake estimates for adults across European countries (Table 9).

7.4.2.3. Consumption of food supplements

Additional intake of HBCDDs could also be caused by high consumption of products for special nutritional use, in particular 'Supplements containing special fatty acids (e.g. omega-3, essential fatty acids)'. These types of supplements are mainly based on fish oil derived from the tissues of oily fish. Fish liver is an important primary source for the preparation of supplements, and in particular cod liver, sold as liquid oil or capsules.

Only 10 samples were reported in the food group 'Products for special nutritional uses' and they were described as 'Supplements containing special fatty acids (e.g. omega-3, essential fatty acids)' (FoodEx level 3; data not reported). As summarised in Table 5, only the individual stereoisomers were analysed therefore LB and UB mean of the sum of the three stereoisomers could be calculated aiming at estimating the additional intake of HBCDDs from food supplements. Among those samples, three were described as bottled fish oil and seven as fish oil in capsules. Despite the high proportion of non-detects across the stereoisomers, the mean LB and UB were calculated and resulted to be 3.19 and 3.56 ng/g for bottled fish oil and 0.36 and 1.14 ng/g fish oil in capsules, respectively.

In order to cover the worst case scenario, a maximum daily consumption of 15 mL of fish oil or 4 g of fish oil as capsules for the dietary exposure estimate to the sum of the HBCDDs stereoisomers was assumed (see Chapter 6.2). Dietary exposure estimates were made assuming a body weight of 60 kg.

Although the estimation is based on only three samples of supplements, the highest dietary exposure to HBCDDs for consumers of 'Supplements containing special fatty acids (e.g. omega-3, essential fatty acids)' is due to consumption of fish oil in its liquid form with up to 0.9 and 1.9 ng/kg b.w. per day, respectively for LB and UB. In the case of consumption of fish oil as capsules the exposure estimates are 0.1 and 1.1 ng/kg b.w. per day for LB and UB, respectively.

7.4.2.4. Vegetarians

HBCDDs are persistent and lipophilic compounds with low water solubility that bioaccumulate in the food chain. Thus, consumption of food of animal origin represents the main route of human dietary exposure to HBCDDs. Since uptake of HBCDDs by plants from soil is low, the contamination of food of plant origin is generally of minor importance. This is substantiated by the occurrence data on HBCDDs in food samples of plant origin submitted by several European countries to EFSA which were almost completely below LOD/LOQ. Consequently, it can be assumed that the dietary HBCDD exposure for vegetarians is even lower than that for people consuming a mixed diet.

7.4.3. Summary of dietary sources of human exposure to HBCDDs

A summary of the dietary sources of HBCDDs for different groups of the population is shown in Table 14.

Occurrence data for 'Food for infants and small children' was limited and not considered adequate to represent products consumed by infants below one year of age and toddlers (e.g. infant formula and follow-up formula). Additionally, considering also the restricted number of consumption data for infants (only two dietary surveys available), the CONTAM Panel decided that it was not appropriate to assess dietary exposure to HBCDDs for infants and toddlers.

Chronic dietary exposure to HBCDDs was therefore assessed only for the following age classes as defined in the dietary surveys included in the Comprehensive Database: 'Other children', 'Adolescents', 'Adults', 'Elderly' and 'Very elderly'.

Additionally, following the occurrence data analysis and based on the composition of the technical HBCDDs mixtures, occurrence in the environment and available data on toxicity, the CONTAM Panel decided to focus the total dietary exposure assessment of the present opinion on the total HBCDD for 'Fish and other seafood' and the sum of the HBCDDs stereoisomers individual level (α-, β- and γ-HBCCD) for the food groups of 'Eggs and egg products', 'Milk and dairy products', 'Meat and meat products' (as described in Table D1, Appendix D).

Table 14: Overview of HBCDD dietary exposure estimates for different groups of the population and different food groups.

Exposed population	**Minimum LB and maximum UB dietary exposures estimates to HBCDDs across European dietary surveys (ng/kg b.w. per day)**			
	Average consumers		**High consumers (95th percentile)**	
	Minimum LB	Maximum UB	Minimum LB	Maximum UB
Breast-fed infants	0.60-142[(a)]		0.90-213[(b)]	
Other children	0.15	1.85	0.8	4.46
Adolescents	0.09	1.06	0.47	2.68
Adults	0.09	0.99	0.39	2.07
Elderly	0.06	0.58	0.32	1.51
Very elderly	0.06	0.54	0.27	1.26
High and frequent fish consumers (total HBCDD)	-	-	1.34	2.76
Consumers of fish liver once a week (total HBCDD)	-	-	1.03	1.94

(a): Considering average human milk consumption (800 mL) and the reported range for total HBCDD in human milk results from the literature (Table 8).

(b): Considering high human milk consumption (1,200 mL) and the reported range for total HBCDD in human milk results from the literature (Table 8).

The age group with the highest mean dietary exposure to HBCDDs across the European dietary surveys is children from three to ten years old ('Other children') with minimum LB and maximum UB of 0.15 and 1.85 ng/kg b.w. per day, respectively, for average population, and of 0.8 and 4.46 ng/kg b.w. per day, respectively, for high consumers. Dietary exposure estimates for 'Adolescents' are 0.09 and 1.06 ng/kg b.w. per day for average population, and 0.47 and 2.68 ng/kg b.w. per day for high consumers, respectively for minimum LB and maximum UB across European countries.

Dietary exposure estimates to HBCDDs in the case of 'Adults' are almost half of those assessed for 'Other children', with minimum LB and maximum UB estimates across European countries for average consumers of 0.09 and 0.99 ng/kg b.w. per day, and for high percentiles consumers 0.39 and 2.07 ng/kg b.w. per day. Lower exposure was estimated for older population groups with minimum

LB and maximum UB for average population of respectively 0.06 and 0.58 ng/kg b.w. per day for 'Elderly' and 0.06 and 0.54 ng/kg b.w. per day for 'Very elderly'. For high consumers, the minimum LB and the maximum UB dietary intake of HBCDDs across European dietary surveys are respectively 0.32 and 1.51 ng/kg b.w. per day for 'elderly' and 0.27 and 1.26 ng/kg b.w per day for 'Very elderly'.

Individuals that might consume fish every day (2.6 g/kg b.w. per day) and weekly consumers of fish liver were considered as specific populations exposed to an additional intake of HBCDDs. For high and frequent fish consumers the mean dietary exposure to HBCDDs ranges from 1.34 to 2.76 ng/kg b.w. per day (minimum LB and maximum UB, respectively). Whereas in the case of fish liver consumers, the mean dietary exposure to HBCDDs ranges from 1.03 to 1.94 ng/kg b.w. per day (minimum LB and maximum UB, respectively).

Consumers of supplements containing special fatty acids (e.g. omega-3, essential fatty acids) were also considered exposed to an additional intake of HBCDDs. Nevertheless, due to the limited occurrence data available, the HBCDDs intakes calculated should be considered only as rough estimates. Assuming a maximum daily consumption of 15 mL of fish oil or 4 g of fish oil as capsules, the highest dietary exposure to HBCDDs was estimated for consumption of fish oil in its liquid form with values of 0.9 and 1.9 ng/kg b.w. per day, respectively for minimum LB and maximum UB. In the case of consumption of fish oil as capsules the exposure estimates are 0.1 and 1.1 ng/kg b.w. per day for minimum LB and maximum UB, respectively.

7.5. Previously reported data on dietary HBCDD intake

Few studies have been found in the literature reporting the dietary intake of HBCDDs in European countries (Table 15). Comparison between studies should be done carefully due to the use of different methodologies (sampling methods and food consumption data), food groups covered and stereoisomers analysed (total HBCDD or individual stereoisomers).

Dirtu and Covaci (2010) estimated the human exposure to total HBCDD through consumption of food of animal origin in Eastern Romania. A total of 71 food samples including meat products, dairy products, vegetable oil and eggs were analysed by GC-MS and combined with official recommendation of the monthly food consumption. Fish samples were not included in the food basket collected. Total HBCDD was below the LOQ in all samples analysed. Assigning non-quantified levels to half the numerical value of the LOQ (medium bound, MB), the estimated median dietary intake was 77 ng per day for adults and 47 ng per day for toddlers (6-24 months).

Goscinny et al. (2011) estimated the dietary intake of the adult Belgian population through the analysis of 42 composite samples from five major food groups including milk and dairy products, meat and meat products, eggs, fish and fishery products and another group that comprised cakes and vegetable oils among others (miscellaneous group). The food consumption data came from the Belgian national food consumption survey of 2004. Stereoisomer-specific analysis was performed. The mean MB dietary exposure was 0.99 ng/kg b.w. per day for the sum of α-, β- and γ-HBCDD. The meat group was the highest contributor (43 %) to the estimated intake, followed by the miscellaneous group (27.6 %) and dairy products (22 %). Fish and fishery products, although being the one showing the highest concentrations, accounted only 7.1 % to the estimated intake due to the low consumption of this food group in Belgium. On a stereoisomer-specific basis, γ-HBCDD showed the highest mean intake for all food groups (0.66 ng/kg b.w. per day) followed by α- (0.25 ng/kg b.w. per day) and β-HBCDD (0.08 ng/kg b.w. per day).

In Sweden, Törnkvist et al. (2011) estimated the dietary intake of total HBCDD from samples from a market-basket study carried out in 2005. Five food groups were considered: fish, meat, dairy products, eggs and fats and oils. The estimated mean intake was 10.2 ng per day (0.14 ng/kg b.w. per day). Fish contributed most to the estimated intake (65 %) followed by dairy products (24 %) and meat (10 %).

The UK Food Standards Agency (FSA, 2006) estimated the dietary intake of α-, β- and γ-HBCDD for UK consumers by analysing 19 composite food samples collected for the 2003 and 2004 total diet studies (TDS). The UB estimated average adult intake from the whole diet was 2.9, 1.5 and 1.6 ng/kg b.w. per day for α-, β- and γ-HBCDD, respectively (5.9 ng/kg b.w. per day for the sum of the three stereoisomers). Meat products followed by fish were the food groups contributing most to the intake. Fernandes et al., (2008) analysed Scottish individual samples of mussels, oysters and scallops for α-, β- and γ-HBCDD and estimated the total dietary intake using for the non-fish part of the diet from the average dietary intakes reported by FSA (2006). The UB dietary intake was estimated to be 5.9-7.9 ng/kg b.w. per day.

Roosens et al. (2009) estimated the exposure via food and dust for 16 Belgian adults. Duplicate diet samples (n=165) were collected in 2007 and analysed for α-, β- and γ-HBCDD. The mean (range) dietary exposure estimate was 7.2 (1.2-20) ng per day. The same authors assessed the human exposure to HBCDDs (sum of α-, β- and γ-HBCDD) through dust, soil, air and food, including human milk for the Flemish population in Belgium (Roosens et al., 2010b). Food samples analysed were grow and produced in Flanders, and these data were complemented with literature values. Median intake for adults was in this case 1.1 ng/kg b.w. per day.

Knutsen et al., (2008) estimated the dietary exposure to HBCDD (sum of α-, β- and γ-HBCDD) in a group of Norwegians (n=184) with a wide range of seafood consumption by use of a food frequency questionnaire covering the previous year and occurrence data from national food monitoring surveys. The mean (range) LB dietary exposure was estimated at 0.33 (0.06-1.35) ng/kg b.w. per day, while the mean UB estimate was 1.3 ng/kg b.w. per day. Oily fish species were the main contributor to the daily intake, followed by meat, hen eggs and dairy products. The authors noted that the exposure calculations should be interpreted with care and that it might not be representative for the general population in Norway, but to show the range of possible dietary exposures among Norwegians with average and high consumption of fish and other seafood.

In The Netherlands, the dietary exposure to total HBCDD was calculated through the analysis of food samples (n=91) including dairy products, meat, animal fat, eggs and vegetable oils (Winter-Sorkina et al., 2003). The consumption data was from the third Dutch National Food Consumption survey. The mean MB dietary exposure was estimated at 2.9 ng/kg b.w. per day (1.5 ng/kg b.w. per day for LB). The authors noted that the limited number of samples analysed (n=91) and the high percentage of non-detects might have introduced uncertainties in the exposure estimates.

Taking into account the differences in methodology and way of reporting among studies, the dietary exposure estimates reported in the literature for different European countries range from 0.1 to 7.9 ng/kg b.w. per day considering the sum of α-, β- and γ-HBCDD and total HBCDD. Meat and fish were in general the food groups contributing most to the dietary intake. In one study where the dietary intake was estimated for the 3 stereoisomers, γ-HBCDD showed the highest intake (0.66 ng/kg b.w. per day). The intakes currently estimated in the present opinion for both average (0.09 and 0.99 ng/kg b.w. per day, for minimum LB and maximum UB, respectively) and high adult consumers (0.39 and 2.07 ng/kg b.w. per day, for minimum LB and maximum UB, respectively) are in the same range as those reported in the literature.

Table 15: Dietary intake to HBCDDs reported in the literature for different European countries.

Country, year	Units	Estimation	α-HBCDD	β-HBCDD	γ-HBCDD	Sum of HBCDD	Total HBCDD	Reference
Belgium, 2007	ng per day ng/kg b.w. per day	(a)	n.r.	n.r.	n.r.	7.2 (1.2-20)[(b)] 0.10[(c)]		Roosens et al., 2009
Belgium, 2008	ng/kg b.w. per day	(d)	n.r.	n.r.	n.r.	1.1		Roosens et al., 2010b
Belgium, 2008	ng/kg b.w. per day	LB MB UB	0.25	0.08	0.66	0.8 0.99 1.18		Goscinny et al., 2011
The Netherlands	ng/kg b.w. per day	MB LB					2.9 1.5	Winter-Sorkina et al., 2003
Norway, 2002-06	ng/kg b.w. per day	LB UB	n.r.	n.r.	n.r.	0.3 (0.06-1.35)[(b)] 1.3[(b)]		Knutsen et al., 2008
Romania, 2007 (Iasi)	ng per day	MB					77	Dirtu and Covaci, 2010
Sweden, 2005	ng per day ng/kg b.w. per day	MB					10.2 0.14	Tornkivst et al., 2011
UK, 2003-04	ng/kg b.w. per day	UB	2.9	1.5	1.6	5.9		FSA, 2006
UK, 2006	ng/kg b.w. per day	UB	n.r.	n.r.	n.r.	5.9-7.9		Fernandes et al., 2008

n.r.: not reported; b.w.: body weight; LB: lower bound: MB: medium bound; UB: upper bound.
(a): Concentrations below LOQ were replaced with *f**LOQ, with *f* being the fraction of samples above the LOQ.
(b): Mean (range).
(c): As reported by Roosens et al., 2010b.
(d): Per congener, an *f*-value, representing the number of measurements above LOQ, was calculated. Values below LOQ were replaced by *f**LOQ. When 0 % of the measurements for a specific congener were above LOQ, the *f*-value for this congener was 0, while the *f*-value equalled 1 when 100 % of the measurements were above LOQ.

7.6. Non-dietary exposure

Non-dietary human exposure to HBCDDs can occur via inhalation of gas-phase HBCDDs and HBCDDs on particles as well as oral intake of house dust. Such exposure can occur in homes as well as in work places. No reports could be identified which indicate that dermal exposure should be of any importance for the total human exposure.

Based on the level of total HBCDD in dust, Abdallah et al. (2008b) identified dust ingestion as an important pathway of exposure to HBCDDs for the UK population. Average dust ingestion was estimated to constitute 23.9 % of total exposure to HBCDDs for adults and 62.6 % for toddlers. High dust intake scenarios (95th percentile) resulted in values of 57.9 % and 91.5 %, respectively. Inhalation was found to be a minor exposure pathway to HBCDDs contributing 1.2 % or less in all scenarios.

In another study Abdallah and Harrad (2009) estimated human average exposure to HBCDDs via dust at average level ingestion to be about 144 ng per day. At a high level of dust ingestion this was about 361 ng per day. Considering a body weight of 60 kg these figures results in daily intakes of 2.4 and 6 ng/kg b.w. The authors also found, that despite shorter exposure duration, car dust accounted for a higher exposure contribution (17 %) compared to office dust (13 %).

Harrad et al. (2010) performed exposure estimates of children (age range 1-6 years) via dust ingestion under 3 exposure scenarios. One 'low-end' scenario where the child ingests 50 mg dust per day contaminated at the 5th percentile concentration, one 'typical' scenario where 50 mg dust per day contaminated at the median concentration is ingested, and one 'high-end' scenario where the child ingests 200 mg dust per day contaminated at the 95th percentile concentration. Assuming a body weight of 20 kg the exposure through dust in homes, classrooms and cars was estimated to be 0.55, 5.9 and 330 ng/kg b.w. for the three scenarios, respectively. The contribution from classrooms to the total exposure through dust was 34 %, 35 % and 22 %, respectively.

Harrad and Abdallah (2011) estimated the total daily HBCDD exposure to UK adults and toddlers from car dust to be 7.7 and 19.3 ng, respectively. The authors estimated that the contribution via ingestion of car dust to the overall daily exposure to total HBCDD was around 2 % for adults and around 6 % for toddlers.

Roosens et al. (2010b) calculated, based on analyses of dust from 43 homes and 10 offices in Belgium, the average daily intake of HBCDDs through dust to be 0.67 and 0.053 ng/kg b.w. for children (<1 year) and adults, respectively. These daily intakes are much lower compared to those reported from UK (Harrad et al., 2008; Abdallah et al., 2008a, b). The latter studies used a median dust ingestion that was some three times higher than that in the Belgian study. This fact can however not totally explain the differences between these findings.

In summary, the available studies indicate that the daily non-dietary exposure, mainly through dust in homes, offices, schools, cars and public environment can substantially contribute, and in some cases even dominates the total human exposure to HBCDDs, especially for toddlers and children.

8. Hazard identification

The CONTAM Panel noted that the number of available studies on HBCDDs published in peer-reviewed journals is relatively small, and limited information is available from other sources which the Panel could not verify for themselves.

8.1. Toxicokinetics

Only few studies have been published on the toxicokinetics of HBCDDs in experimental animals.

In a 90-day repeated dose toxicity study with rats given 1,000 mg/kg b.w. per day of technical HBCDD, it was found that the concentrations in adipose tissue of the α-stereoisomer were much higher than those of the β- and γ-isomers throughout the study period (Chengelis, 2001, cited in ECB, 2008). The relative percentage of the stereoisomers measured in the rats (α-: 65-70 %; β-: 9-15 % and γ-: 14-20 %) was markedly different from those in the HBCDD formulation used (α-: 8.9 %; β-: 6.6 % and γ-: 84.5 %).

In a 28-day oral dose toxicity study, van der Ven et al. (2006) administered technical HBCDD (10.3 % α-HCBDD, 8.7 % β-HBCDD and 81.0 % γ-HBCDD) by daily gavage to male and female rats, with doses 0, 0.3, 1, 3, 10, 30, 100 or 200 mg/kg b.w. per day. α- and γ-HBCDD were analysed in the liver and adipose tissue at the end of the experiment, showing a dose dependent increase, with a plateau at approximately 100 µg/g liver lipid in males for both enantiomers at the three highest doses. The hepatic HBCDD concentrations were higher in females than in males over the entire dose range (on average about 5 times). The average ratio γ-/α-HBCDD, which was approximately 8 in the administered HBCDD technical mixture, was found to be 4 in females and 3 in males from the 3 mg/kg dose group and decreased with dose in both sexes. Total average concentrations found in adipose tissue from females and males of the 10 mg/kg b.w. dose group were 380 and 85 µg/g, respectively. These levels are 2.4 and 1.4 times higher than in liver fat.

In a one-generation reproduction study performed in Wistar rats (van der Ven et al., 2009), the animals were fed a diet containing a HCBDD technical mixture (α-HCBDD 10.3 %, β-HBCDD 8.7 % and γ-HBCDD 81.0 %). HBCDD mixture was incorporated into the diet at different concentrations in order to obtain a dietary exposure of 0, 0.1, 0.3, 1, 3, 10, 30 or 100 mg/kg b.w. per day. Exposure period for parental male rats was 10 weeks whereas females were exposed for 8 weeks (starting 2 weeks prior to mating). The hepatic total HBCDD concentrations measured in F1 animals at the termination of the exposure period (11 weeks of age) were higher in females compared to males over the entire dose range. The ratio γ/α was approximately 8 in the administrated technical HBCDD. At a dose of 10 mg/kg b.w. the ratio in the lipid fraction of liver was 0.9 for females and 1.4 for males. These ratios declined to 0.04 and 0.2 in females and males, respectively, at the highest dose (100 mg/kg b.w.) Measurable concentrations of β-HBCDD were only found in females of the two highest dose groups, and in males of the highest dose group. In the highest dose group, average concentrations of sum α-, β- and γ-HCBDD were about 930 and 170 µg/g liver lipid in females and males respectively.

Recently, Szabo et al. (2010, 2011) investigated the disposition of HBCDDs in female C57BL/6 mice administered a single oral dose (3, 10, 30 or 100 mg/kg) of either [^{14}C]γ-HBCDD or [^{14}C]α-HBCDD. In both cases, gastro-intestinal absorption was found to be ≥ 85 % of the administered dose (3 mg/kg). For [^{14}C]γ-HBCDD, one day or four days following the administration, highest residue concentrations, based on radioactivity measurements, were found in adrenals, followed by liver, skin, muscle, kidney, adipose tissue, blood and brain. With respect to [^{14}C]α-HBCDD, concentrations observed one day after dosing were in the following order: liver > muscle > adipose tissue > blood > brain > kidney (levels in adrenals not reported), whereas adipose tissue had the highest levels after four days, followed by liver, skin, blood and muscle. Four days after oral administration of equivalent doses (3mg/kg), α-HBCDD residue levels were approximately 60 fold higher than γ-HBCDD levels in the liver, and nearly 500 fold higher in the adipose tissue. In addition, in mice repeatedly dosed for 14 days with α- or γ-HBCDD, a significant accumulation of residues was observed for α-HBCDD, but not for γ-HBCDD. Elimination of α-HBCDD and γ-HBCDD was primarily in feces and to lesser extent in urine. [^{14}C]γ-HBCDD was rapidly metabolized. Mono- and dihydroxylated pentabromocyclododecene metabolites were found in fecal extracts. *In vivo* conversion was observed of the γ-isomer to the β-isomer in liver and brain tissues, and to the α- and β-isomer in fat and feces. Elimination, both whole-body and from individual tissues, was biphasic. Initial half-lives were approximately 1 day,

whereas terminal half-lives were up to 4 days. Kinetics of elimination of [^{14}C]α-HBCDD were substantially slower than for the γ-isomer. A terminal half-life of 17 days was measured in the adipose tissue of mice treated by α-HBCDD whereas it was of 2-6 days for different tissues, including adipose tissue (3.6 days), for γ-HBCDD. Several unidentified polar metabolites were detected in bile, serum and urine, but in contrast to γ-HBCDD, no stereoisomerisation products were detected in excreta and tissues.

Brandsma et al. (2009) identified several metabolites from rats exposed to 30 and 100 mg/kg b.w. per day HBCDD technical mixture for 28 days. Four different types of hydroxylated HBCDD metabolites were extracted from adipose tissue, liver, lung and muscle: the monohydroxy metabolites of HBCDD, pentabromocyclododecene, tetrabromocyclododecene and dihydroxy-HBCDD. Debromination of HBCDD to pentabromocyclododecene was also reported.

Geyer et al. (2004) estimated terminal elimination half-life in humans for HBCDDs (sum of α-, β-, γ-stereoisomers) from extrapolation from measured half-life in the fat of rats, and from calculations based on body burden and daily intake in non-occupationally exposed adult humans. Both methods resulted in a half-life value of 64 days (range 23-219 days).

The available toxicokinetics data, suggest that orally administered HBCDD is extensively absorbed and rapidly distributed in different tissues, with some differences observed between γ- and α-isomer. In contrast to γ-HBCDD, α-HBCDD was found to concentrate in adipose tissue. Debromination and hydroxylation seem to be the major metabolic pathways for HBCDD, but stereoisomerisation of γ-isomer to α- and β-isomers was observed in mice treated with γ-HBCDD. No stereoisomerisation was reported after exposure to α-HBCDD. Elimination half-lives of HBCDD stereoisomers in female mice, based on concentrations in adipose tissue, vary from 3-4 days for the γ-isomer, to 17 days for the α-isomer. The half-life was estimated to be 64 days (range 23-219 days) in humans for HBCDDs (sum of α-, β- and γ-HBCDD).

8.2. Biomarkers of exposure

The studies reporting levels of HBCDDs in human samples other than human milk are scarce and deal mainly with serum or adipose tissue samples. Total HBCDD was usually reported and half of the studies reported concentrations for the individual stereoisomers (α-, β- and γ-HBCDD) (Table 16).

Weiss et al. (2006) reported the levels of the 3 main HBCDD stereoisomers in 50 serum samples from women from a previously established cohort of wives and ex-wives of professional fishermen from the Swedish east coast. Total HBCDD concentrations (sum of α-, β- and γ-HBCDD) ranged from <0.24 to 3.4 ng/g fat (<0.37 to 5.4 pmol/g fat), with α-HBCDD being the predominant isomer. Γ-HBCDD was found to contribute between 1-3 % to the total HBCDD concentration. Enantioselective analysis was also performed and (–)α-HBCDD was identified as the dominant enantiomer.

Thomsen et al. (2007) investigated serum HBCDD concentrations of 10 workers at an industrial plant producing expandable polystyrene with added HBCDDs as a flame retardant. Two serum samples were collected for each worker: first sampling was performed during the first production period and the second sampling about 6 weeks after the end of the manufacturing of the HBCDD-containing products. The median serum concentration of total HBCDD for all samples was 101 ng/g fat (92 and 117 ng/g fat for the first and second sampling, respectively). The authors also analysed the serum levels of 10 persons expected to have background exposure to HBCDDs as reference group. None of the samples in the reference group showed HBCDD levels above the LOD (~1 ng/g fat). The stereoisomer specific analyses of the serum samples of the first sampling showed that α- and γ-HBCDD were detected in all samples with relative contributions to total HBCDD 60 and 39 %, respectively. β-HBCDD was detected in two out of the 10 samples and contributed about 4 % to the total HBCDD content.

In another study, Thomsen et al. (2008) measured the serum concentration of HBCDDs in a group of high consumers of fish from the contaminated Lake Mjøsa (Norway) to investigate possible relationships between the serum concentration and self-reported fish intake. The study group comprised 66 persons (41 men and 25 women). HBCDDs were detected in 49 out of the 66 samples with median concentration (range) of 4.1 (<LOQ-52) ng/g fat and 2.6 (<LOQ-18) ng/g fat for men and women, respectively.

Meijer et al. (2008) reported the levels of HBCDDs (sum of α-, β- and γ-HBCDD) in serum of pregnant women at the 35th week of pregnancy from the Groningen-infant-mother cohort and in cord serum of a number of their infants. Concentrations in maternal serum (n=69) were above the LOD except in one sample, while in cord serum 5 out of the 12 samples analysed were above the LOD. In maternal serum median concentration (range) of total HBCDD was 0.7 (ND-7.4) ng/g fat, in cord serum (n=12) it was 0.2 (0.2-4.3) ng/g fat.

Kalantzi et al. (2011) analysed serum samples from Greek male and female computer clerks working full time with computers (n=30) and from a control population (n=31). HBCDDs were detected in 43 out of the 61 serum samples (70 %). The median concentration (range) was 1.32 (0.49-38.8) ng/g fat. Differences between the computer clerks and the control population were not reported in the publication. Females from both groups had lower HBCDD concentrations than males (median 0.71 ng/g and 1.44 ng/g fat, for females and males, respectively).

Lignell et al. (2011) studied the temporal trends of HBCDDs in blood serum from primiparae mothers from Uppsala (Sweden) between 1996 and 2010. A total of 413 individual samples were obtained and 3 pooled serum samples for each sampling year were analysed. The concentrations of HBCDDs were below the LOQ in more than 70 % of the samples. The mean concentration across 1996-2010 was 0.28 ng/g fat. After linear regression analysis, the authors reported that HBCDDs decreased significantly in serum during the study period. This decrease reported by the authors is in contrast to the somewhat increase observed in human milk samples (see Chapter 5.2.2).

Antignac et al. (2008) measured the levels of HBCDD stereoisomers in maternal adipose tissue (n=26). α-HBCDD was identified in approximately 50 % of the samples, with concentrations ranging from 1 to 3 ng/g fat. Three of the samples showed much higher values (6-12 ng/g fat). β- and γ-HBCDD were not detected in any of the samples. Maternal and umbilical serum samples were also included in the study design although the restricted sample amount did not allow their analysis with the proposed LC-MS/MS methodology.

Pulkrabová et al. (2009) reported the levels of HBCDDs in adipose tissue samples (n=98) obtained by liposuction of people living in the Czech Republic. The analyses were carried out by GC-ECNI-MS and therefore quantification of the individual HBCDD stereoisomers was not possible. HBCDDs were only detected in 15 % of the samples analysed with a mean concentration of 1.2 ng/g fat (median: <0.5 ng/g fat, 5 %-95 % percentile: <0.5-7.5 ng/g fat).

Summarising, the median concentration of total HBCDD in serum and adipose tissue samples was in general not higher that 3 ng/g fat, except when considering occupational exposure where the levels were reported to be up to 101 ng/g fat. α-HBCDD was found to be the dominating isomer in serum and adipose tissue samples, while β- and γ-HBCDD were not detected or contributed only 1-3 % to the total. In contrast, in serum samples from workers exposed to HBCDDs, the contribution of γ-HBCDD was reported to be much higher (39 %) pointing to direct exposure to the technical HBCDD where γ-HBCDD is predominant (about 78 %). Therefore, higher levels of γ-HBCDD than α-HBCDD might indicate recent exposure to technical HBCDD.

Table 16: Levels of HBCDDs (ng/g fat) in human matrices (blood [B] and adipose tissue [AD]) from different European countries reported in the literature.

Country, Year	Sample	N	α-HBCDD	β-HBCDD	γ-HBCDD	Sum of HBCDD	Total HBCDD			Reference
							mean	median	range	
Czech Republic, 2007	AD	98	-	-	-	-	1.2	< 0.5	<0.5-7.5	Pulkrabová et al., 2009
France, 2005	AD	26	1-12	< LOD	< LOD	n.r.	-	-	-	Antignac et al., 2008
Greece, 2007	S	61[a]	-	-	-	-	n.r.	1.32	0.49-38.8	Kalantzi et al., 2011
Norway, 2004-2005	S	41 men[c] 25 women[c]	-	-	-	-	9.6 3.7	4.1 2.6	< LOQ-52 < LOQ-18	Thomsen et al., 2008
Scandinavia	S	10[d] 10 (control)	n.r.	n.r.	n.r.	101 < 1	-	-	-	Thomsen et al., 2007
Sweden	S	50[b]	n.r.	n.r.	n.r.	< 0.24-3.4	-	-	-	Weiss et al., 2006
Sweden, 1996-2010	S	36[e]	-	-	-	-	0.28	-	-	Lignell et al., 2011
The Netherlands, 2001-2002	S (maternal) S (cord)	69 12	-.	-	-	-	-	0.7 0.2	<LOD-7.4 0.2-4.3	Meijer et al., 2008

N: number of samples; n.a.: not analysed; n.r.: not reported.
(a): Greek male and female computer clerks working full time with computers (n=30) and from a control population (n=31).
(b): Cohort of wives and ex-wives of professional fishermen from the Swedish east coast.
(c): High consumers of fish from the contaminated Lake Mjøsa (Norway).
(d): Workers at an industrial plant producing expandable polystyrene added HBCDDs as a flame retardant.
(e): Pooled samples.

8.2.1. Relation between exposure estimates and levels in humans

Only one study investigating the relation between exposure and concentrations in blood was identified. Roosens et al. (2009) found that concentrations in bedroom dust, but not concentration in diet measured in duplicate diets for one week, correlated with HBCDD concentrations in serum in a study on 16 Belgian adults. The magnitude of the calculated exposure from diet and dust was quite similar, and the authors state that a possible reason for the lack of correlation between dietary exposure and serum levels could be that the HBCDD concentration in duplicate diets did not reflect the long term dietary intake of HBCDD.

8.3. Toxicity

All *in vivo* toxicity studies available were carried out with technical HBCDDs mixtures containing more than one stereoisomer (Table 17). It should be noted that the isomer profile found in food differs substantially from the one in technical mixtures used in toxicology studies.

8.3.1. Acute toxicity

Acute toxicity from exposure to technical HBCDD is very low, and an LD_{50} value has not been determined. The oral lethal dose is more than 20 g/kg b.w. in rats and more than 40 g/kg b.w. in mice (ECB, 2008).

8.3.2. Sub-chronic and chronic toxicity

Main targets in sub-chronic and chronic toxicity studies were the liver, thyroid hormone homeostasis and the reproductive, nervous and immune systems.

8.3.2.1. Endocrine-related effects

In a 28-day feeding study Sprague-Dawley rats received 0, 1.0, 2.5 or 5.0 % (approximately equivalent to 0, 940, 2,400 or 4,700 mg/kg b.w. per day, respectively) HBCDDs (Hexabromid-S), in the feed mixed with 1 % olive oil (Zeller and Kirsch, 1969, as cited in ECB (2008)). In the high dose group females exhibited signs of inhibited oogenesis and sparse ripening follicles in the ovaries. In males, differentiation of sex organs and spermiohistogenesis were normal, however, the epididymes were small.

In a two-generation reproductive toxicity study rats were administered a diet containing HBCDDs mixed in ground food from 10-week pre-mating period and continuing through the mating, gestation and lactation periods for two generations (Ema et al., 2008). The concentrations of HBCDDs in the diet were 150, 1,500 or 15,000 mg/kg, corresponding to 10.2, 101 and 1,008 mg/kg b.w. per day in F0 males, 14.0, 141 and 1,363 mg/kg b.w. per day in F0 females, 11.4, 115 and 1,142 mg/kg b.w. per day in F1 males, and 14.3, 138 and 1,363 mg/kg b.w. per day in F1 females. A dose-dependent decrease (8-14 %) in fertility index was observed in F0 and F1 generations, but was statistically significant only in F0. In the mid and high dose pup mortality during lactation was observed in the F2 generation. This was statistically significant only in the high dose group. Histopathological examination of the ovaries of F1 females revealed a decreased number of primordial follicles at 1,500 and 15,000 mg/kg diet. No concurrent adverse effects on reproductive parameters in F1 dams, or on the numbers of implantations or F2 pups delivered were noted. F1 parent rats were subjected to a single breeding trial and the authors suggested that a continuous breeding study of HBCDD may be needed to clarify the reproductive toxicity of HBCDD, especially the adverse effects of HBCDD on the reproductive life span. The NOAEL of this study is 150 mg/kg diet (10 mg/kg b.w. per day) based on decrease in fertility index in F0 animals and the decrease in the number of primordial follicles in F1 females.

Table 17: Summary of the major outcomes of toxicological studies of HBCDDs in experimental animals.

Compound	Purity/impurities	Species	Exposure and level(s)	Outcome	LOEL	NOEL	BMD(L)	Reference
HBCDD	> 98 %	NMRI mice	0.9 or 13.5 mg/kg b.w. via gavage PND10	At 0.9 mg/kg hypoactivity at 3 months decreased habituation impaired learning	0.9 mg/kg b.w.			Eriksson et al., 2006
Hexabromid S	6.3 % α-, 9.1 % β-, 76.9 % γ-HBCDD, 1 % tetrabromo-cyclododecane, 0.2 % isobutanol, 6.5 % of unknowns	Sprague-Dawley rats	28-day feeding: 940, 2,400 or 4,700 mg/kg b.w. per day in feed	- Inhibited oogenesis - Dose dependent thyroid hyperplasia and activity, hypotrophy epididymes	940 mg/kg per day			Zeller and Kirsch, 1969 (as cited in ECB, 2008)
Technical HBCDD	10.3 % α-, 8.7 % β- and 81.0 % γ-HBCDD, respectively	Wistar rats	28-day study: 0, 0.3, 1, 3, 10, 30, 100, 200 mg/kg b.w.	Induction of hepatic drug metabolism, , more pronounced in females	CYP2B: 30 (female) 100 (male) CYP3A: 3 (female) 30 (male)	CYP2B: 10 (female) 30 (male) CYP3A: 1 (female) 10 (male)		Germer et al., 2006
Technical HBCDD	10.3 % α-, 8.7 % β- and 81.0 % γ-HBCDD, respectively	Wistar rats	28-day study: 0.3, 1, 3, 10, 30, 100 and 200 mg/kg b.w. per day by gavage	- Increased thyroid weight - Decreased total T4 - Increased TSH immunostaining and weight pituitary - Decreased splenocyte count - Increased hepatic capacity of T4 UGT (f) - Increased liver weight (f)			- Increased thyroid weight: $BMDL_{10}$ 1.6 mg/kg b.w. per day - T4 decrease: $BMDL_{10}$ 55.5 mg/kg b.w. per day - Pituitary weight: $BMDL_{10}$ 29 mg/kg b.w. per day - Splenocyte count: $BMDL_{20}$ 104 mg/kg b.w. per day - T4 UGT: $BMDL_{10}$ 4.1 mg/kg b.w. per day - Liver weight: $BMDL_{20}$ 23 mg/kg b.w. per day	van der Ven et al., 2006

Table 17: Continued.

Compound	Purity/impurities	Species	Exposure and level(s)	Outcome	LOEL	NOEL	BMD(L)	Reference
HBCDD	Not reported	Mice Female BALB/c	28-day study: 1 % in feed	- Increased RSV infection		Ca. 1700 mg/kg b.w. per day.		Watanabe et al., 2010
Technical HBCDD	90 % (0.7 % tetrabromo-cyclododecane, 0.1 % isobutanol and 9.2 % unknown)	Sprague-Dawley rats	90-day study: 100, 300 or 1,000 mg/kg b.w. per day by gavage	- Increase in relative prostate weight - Thyroid follicular cell hypertrophy - Decrease in serum T4 Increase in TSH	300 mg/kg b.w. per day	100 mg/kg b.w. per day		Chengelis, 2001 (as cited in ECB, 2008)
HBCDD	> 95 %	Sprague-Dawley rats (Crj:CD IGS rats)	Developmental study from GD10 to PND20 and PNW11: 100, 1,000 and 10,000 mg/kg diet	- Reduced number of CNPase-positive oligodendroglia in the cortex - Increased relative thyroid weight in male - Thyroid follicular cell hypertrophy - Decreased serum T3	10,000 mg/kg diet 1,000 mg/kg diet (decrease in T3; increase thyroid weight in male offspring)	100 mg/kg diet 100 mg/kg diet (8.1-21.3 mg/kg by maternal exposure) (decrease in T3; increase thyroid		Saegusa et al., 2009
Technical HBCDD	10.3 % α-, 8.7 % β- and 81.0 % γ-HBCDD	Wistar WU (CBP) rats	One generation reproduction study: 0,1; 0,3; 1; 3; 10; 30 and 100 mg/kg b.w. per day in the feed. Exposure before mating till 11 weeks of age of F1.	- Decrease in testes weight - Increased anogenital distance (PND4) - Delayed vaginal opening - Increased IgG response - Decreased trabecular bone mineral density (females) - Decreased in apolar retinoids			- Testes: $BMDL_5$ 11.5 mg/kg b.w. per day - IgG response: $BMDL_{20}$ 0.46 mg/kg b.w. per day - Bone mineral density: $BMDL_{10}$ 0.056 mg/kg b.w. per day - Retinoids: $BMDL_{10}$ 1.3 mg/kg b.w. per day	van der Ven et al., 2009

Table 17: Continued.

Compound	Purity/impurities	Species	Exposure and level(s)	Outcome	LOEL	NOEL	BMD(L)	Reference
HBCDD	α-, β-, and γ-HBCDDs (10.3, 8.7, and 81.0 %, respectively) with traces of tetra- and pentabromocyclododecane	Wistar WU (CBP) rats	1.43, 4.29, 14.3, 42.9, 143, 429, 1,430 mg/kg in diet (estimated 0.1, 0.3, 1, 3, 10, 30, 100 mg/kg b.w.) before mating to 11 weeks	- BAEP alterations suggestive for cochlear defect - Reduced latency to move after haloperidol			BAEP: 1-6.3 mg/kg Catalepsy: 0.6-4.4 mg/kg	Lilienthal et al., 2009
HBCDD	99.7 % (8.8 % α-, 7.9 % β- and 83.7 % γ-HBCDD)	Crl:CD(SD) rats	Two-generation study: 150, 1,500 and 15,000 mg/kg diet (about 10, 100 and 1,000 mg/kg b.w.)	- Decrease in fertility index in F0 and F1 animals - Decrease in ovary primordial follicles in F1 females - Decreased thyroid follicle size - Increase of thyroid weight in F0 and F1 animals - Decrease in serum T4 in all animals - Increase in serum TSH in F0 and F1 females	1,500 mg/kg diet (100 mg/kg b.w.)	150 mg/kg diet (10 mg/kg b.w.)		Ema et al., 2008

LOEL: lowest-observed-effect level; NOEL; no-observed-effect level; BMD(L): benchmark dose (limit); TSH: thyroid-stimulating hormone; T4: thyroxine; T3: triiodothyronine UGT: UDP glucuronosyltransferase; RSV: respiratory syncytial virus; GD: gestational day; PND: postnatal day; BAEP: brainstem auditory evoked potential; PNW: postnatal week.

In a one generation reproduction study in Wistar rats, in which endocrine parameters were included, animals were exposed to HBCDD mixture (10.3, 8.7 and 81.0 % α-, β- and γ-HBCDD, respectively) dissolved in corn oil and mixed in the standard laboratory diet (van der Ven et al., 2009). Dose levels were 0, 0.1, 0.3, 1, 3, 10, 30 or 100 mg/kg b.w. per day. Parental rats were exposed prior to the mating (10 weeks for males; 2 weeks for females) and was continued through mating, gestation and lactation. F1 offspring were exposed from weaning until final necropsy at 11 weeks age. The effects were assessed in F1 animals. The most sensitive effect on reproductive organs was decrease in weight of the testes with a $BMDL_5$ of 11.5 mg/kg b.w. per day. When the same effect is expressed against concentrations of HBCDDs in liver of F1 the $BMDL_5$ was 52 µg/g liver lipid. Male pups showed increased anogenital distance at postnatal day (PND) 4 ($BMDL_{10}$ 95.6 mg/kg b.w.), but not on PND7 and 21. In female pups a delayed vaginal opening was observed ($BMDL_{10}$ 82.2 mg/kg b.w.). In this study the most sensitive effect was the decrease of the trabecular bone mineral density of the tibia in females in the F1 generation ($BMDL_{10}$ 0.056 mg/kg b.w. per day).

Taken together, from the 2-generation reproductive toxicity study the NOAEL for reduced fertility index and for the reduction of the number of ovarian primordial follicles is 10 mg/kg b.w. per day, whereas from the one generation reproductive study the most sensitive effect on reproductive organs is a decrease in testes weight with a $BMDL_5$ of 11.5 mg/kg b.w. per day. In addition effects on mineral bone density were observed with a $BMDL_{10}$ of 0.056 mg/kg b.w. per day.

8.3.2.2. Teratogenicity

Murai et al. (1985) exposed pregnant Wistar rats via the diet to doses of 0, 7.5, 75 or 750 mg HBCDDs/kg b.w. per day from gestational day (GD) 0 to GD20. Six animals per group were allowed to deliver and the pups were maintained until the week 7. The absolute and relative maternal liver weight was significantly increased at the highest dose. No changes in number of implants, resorptions, live or dead fetuses, or external, visceral or skeletal anomalies were observed in the pups.

Stump (1999) did not observe maternal or fetal toxicity in Charles River rats exposed by gavage to 500 or 1,000 mg HBCDDs/kg b.w. per day on GD6 to GD9.

Although the studies mentioned above did not show any fetotoxicity or teratogenic effects of HBCDDs in rats, increased pup mortality during lactation was statistically significant in the F2 generation at the highest dose level (males 1,008 mg/kg b.w. per day, females1,363 mg/kg b.w. per day) in the two-generation reproductive toxicity study (Ema et al., 2008) (see Chapter 8.3.2.1).

8.3.2.3. Interactions with the thyroid hormone system

Four repeated dose studies (three 28-day studies and a 90-day study), a one-generation study, a two generation reproductive toxicity study and a developmental toxicity study with oral administration of HBCDDs were available for evaluation of effects of HBCDDs on the thyroid hormone system.

In the 28-day feeding study with Sprague-Dawley rats performed by Zeller and Kirsch (1969, as cited by ECB, 2008) (see above) the thyroids of exposed animals showed a dose dependent increase in hyperplasia and activity of the follicular epithelial cells. Serum concentrations of thyroid stimulating hormone (TSH), triiodothyronine (T3) and thyroxine (T4) were not measured. LOEL was 940 mg/kg b.w. per day (lowest dose tested).

In the 28-day study by van der Ven et al. (2006), seven weeks old Wistar rats were administered a HBCDD mixture (10.3 %, 8.7 % and 81.0 % for α-, β- and γ-HBCDD, respectively) dissolved in corn oil at doses 0, 0.3, 1, 3, 10, 30, 100 or 200 mg/kg b.w. per day by gavage. The most marked dose-related effects on thyroid hormone axis were decreased total T4 ($BMDL_{10}$ 55.5 mg/kg b.w. per day), increased TSH immunostaining of the pituitary and pituitary weight ($BMDL_{10}$ 29.0 mg/kg b.w. per day), and increased thyroid weight ($BMDL_{10}$ 1.6 mg/kg b.w. per day equivalent to 43 µg HBCDD/g

liver lipid). Although the increased thyroid weight, according to calculated $BMDL_{10}$, seems to be the most sensitive parameter, the dose response-effect was not clear (at doses up to 30 mg/kg b.w, except at the dose 1 mg/kg b.w. the increase was less than 6 %). These effects were restricted to females and were in line with higher hepatic HBCDD concentrations in females than in males (see Chapter 8.1).

In a 90-days oral toxicity study Sprague-Dawley rats were dosed daily by gavage with 0, 100, 300 or 1,000 mg/kg b.w. per day of technical HBCDD (90 % HBCDDs, 0.7 % tetrabromocyclododecane, 0.1 % isobutanol and 9.2 % unknown) (Chengelis, 2001, as cited in ECB, 2008). Thyroid follicular cell hypertrophy was observed in females at the mid and high dose, and in males at the high dose. Serum concentrations of T4 were decreased in males and TSH was increased in both sexes, with a LOEL of 100 mg/kg b.w. per day.

In the one generation reproduction study in Wistar rats by van der Ven et al. (2009), in which animals were exposed to an HBCDD mixture at target doses from 0 to 100 mg/kg b.w. per day in the diet, no effect on thyroid related parameters (histopathological changes in the thyroid, total T4 and T3) were observed in F1 animals.

In the two-generation reproductive toxicity study by Ema et al. (2008), described in 8.3.2.1, a statistically significant increased incidence of animals with decreased thyroid follicle size was observed in the F0 and F1 mid and high dose groups. Increased relative thyroid weight was observed in high dose F0 and F1 males and in high dose F1 females. Serum T4 levels were decreased in all animals at the high dose, although a statistically significant decrease was observed only in F0 males and females. Serum TSH was increased at all dose levels in F0 females, and at the two highest doses in F1 females. In males, a statistically non-significant increase in TSH was observed at the highest dose. There were no effects on T3 levels in any group.

In a developmental toxicity study Saegusa et al. (2009) administered pregnant Sprague-Dawley rats 0, 100, 1,000 or 10,000 mg/kg diet HBCDDs (purity >95 %), from GD10 until PND20. Dietary dose levels corresponded to about 8.1-21, 81-213 or 800-2,230 mg/kg b.w. per day, respectively, for maternal exposure. Maternal exposure resulted in increased relative thyroid weight at the highest dose. Thyroid follicular cell hypertrophy was observed at 100 and 1,000 mg/kg diet HBCDD. A significant increase in relative thyroid weight was observed only in male offspring on postnatal week (PNW) 11. A statistically significant decrease of serum T3 was observed in offspring at 10,000 mg/kg diet on PND20 and at 1,000 and 10,000 mg/kg diet on PNW11. Serum T4 concentrations were unaltered. A statistically significant increase of serum TSH was observed at 10,000 mg/kg diet on PND20, but not on PNW11.

Taken together, all studies except the one generation study by van der Ven (2009) showed effects on the thyroid hormone system. In the 28-day study by van der Ven et al (2006), the most sensitive effect was increased relative thyroid weight in female rats with a $BMDL_{10}$ value of 1.6 mg/kg b.w. per day, however without a clear dose relationship. The changes in total T4 ($BMDL_{10}$ 55.5 mg/kg b.w. per day), and increased TSH pituitary immunostaining and weight ($BMDL_{10}$ 29.0 mg/kg b.w. per day) were observed at higher doses. These effects were observed only in female rats. The two generation reproductive toxicity rat study (Ema et al., 2008) showed effects on thyroid system with a LOEL of 100 mg/kg b.w. per day in F0 and F1 male animals, whereas the developmental study by Saegusa et al. (2009) indicated a NOEL of 100 ppm (corresponding to 8.1-21.3 mg/kg b.w. per day for maternal exposure), based on decrease in T3 and increase of relative thyroid weight in male offspring.

8.3.2.4. Nervous system

Neurodevelopmental effects following single administration

Eriksson et al. (2006) exposed male NMRI mouse pups to HBCDDs at PND10. A mixture containing three stereoisomers (α-, β- and γ-HBCDD with a relative content of 3 %, 8 % and 89 %, respectively (Fång, 2007)) was prepared from a commercial mixture to a purity of over 98 % (the 2 % of non-HBCDDs were due to tetrabromocyclododecenes) and administered by gavage once at a dose of 0, 0.9 or 13.5 mg/kg b.w. At the age of three months, the mice were assessed for spontaneous behaviour, as well as for learning and memory. Ten mice per group (originating from 3 to 4 litters) were tested for spontaneous behaviour by measuring locomotion (horizontal movement), rearing and total activity (all movements, including stereotypes). The activities were measured for three 20-minute intervals of a 60minute observation period. For all variables, the control animals became habituated, i.e. activity in response to the novelty of the test chamber diminished over time. The animals exposed to HBCDDs exhibited alterations in spontaneous behaviour. Compared to the controls they were hypoactive during the first 20 min of the observation period, while toward the end of the test period they were more active. Associative learning and memory were assessed by a Morris swim maze. Groups of 12-17 male mice (originating from 3 to 4 litters) were tested for the ability to locate a submerged platform in a pool for four consecutive days, and on the fifth day, were tested to find the platform in a changed location in the pool. Five trials were carried out each day. During the acquisition period (days 1-4), both exposed and control mice improved their ability to locate the platform. On the fourth day, the mean latencies of the mice exposed to 13.5 mg/kg b.w. were significantly longer than controls and the group exposed to 0.9 mg/kg b.w. The mice in the lower dose group did not differ significantly from the controls. However, on the fifth day, mice exposed to either 0.9 or 13.5 mg/kg b.w. took significantly longer time to find the new position of the platform. The LOEL of this study was 0.9 mg/kg b.w., the lowest dose tested in the study. The CONTAM Panel noted that the exposure protocol used in this study is not in accordance with the OEDC 426 guideline for developmental neurotoxicity studies that recommends continuous exposure during gestation and lactation to resemble human exposure.

Neurodevelopmental effects following repeated administration

In a two-generation reproduction study by Ema et al. (2008), Crl:CD Sprague-Dawley rats showed significant neurodevelopmental effects in the F1 and F2 offspring at the highest dose only. In male pups from dams of the highest dose group (15,000 mg/kg diet HBCDDs) surface righting reflex time was shorter (F1, tested on PND5). In female pups (F2, tested on PND18) of these dams a lower completion rate for mid-air righting reflex was found. The completion rate of eye opening at PND14 was higher in F1 pups exposed to 1,500 mg/kg diet HBCDDs, and lower in F2 pups exposed to 15,000 mg/kg diet HBCDDs, and in female F2 pups exposed to 1,500 mg/kg diet. In addition, the absolute weight of the brain was decreased in adult rats and in F1 and F2 pups exposed to 15,000 mg/kg diet HBCDDs.

Lilienthal et al. (2009) conducted a dietary study on litter mates from the one-generation study from van der Ven et al. (2009), using technical HBCDDs (Table 16) dissolved in corn oil and mixed in standard laboratory diet. The targeted exposure levels were of 0, 0.1, 0.3, 1, 3, 10, 30 or 100 mg/kg b.w. per day. The exposure was initiated prior to mating (10 weeks for males, 2 weeks for females), and was continued throughout mating, gestation and lactation. Each F0 group consisted of 10 males and 10 females. All F1 litters were housed individually after weaning and maintained on the same diet until 11 weeks of age. The offspring (1 male and 1 female per litter) were assessed for hearing function (brainstem auditory evoked potentials, BAEP) and dopamine-dependent behaviour (haloperidol-induced catalepsy). The authors found significant differences in BAEP (increased thresholds and prolonged latencies of early waves) only in F1 male rats. The overall pattern of the evoked potentials with increased thresholds and prolonged latencies of early waves pointed to an effect on cochlea. Reduced latencies to movement onset in the haloperidol-induced catalepsy were more pronounced in female than in male rats. Analyses with benchmark models revealed $BMDL_5$

values of 0.6-4.4 mg/kg per day for reduced latencies to movement onset in catalepsy and of 0.2-0.9 mg/kg per day for increased thresholds in the BAEP.

Saegusa et al. (2009) exposed pregnant Sprague-Dawley rats to 0, 100, 1,000 or 10,000 mg/kg diet HBCDDs (purity >95 %) from GD10 until PND20. Dietary dose levels corresponded to about 8.1-21, 81-213 or 800-2,230 mg/kg b.w. per day, respectively, for maternal exposure. The offspring were maintained on regular diet until 11 weeks of age and then killed for histological assessment. HBCDDs showed evidence of affecting oligodendroglial development at a dose of 10,000 mg/kg diet.

In conclusion, the few available data show that HBCDDs can affect neurodevelopment. Of particular relevance are the behavioural changes indicating alterations in motor activity and learning abilities reported after a single postnatal administration in mice. The most relevant effects observed after repeated exposure seem to be sex-related with hearing alterations occurring in male rats and changes in haloperidol-induced catalepsy most prominent in females.

8.3.2.5. Immune system

Decreased splenocyte counts ($BMDL_{20}$ 104 mg/kg b.w.) were seen in the 28-day repeated dose study in rats administered HBCDDs by gavage (van der Ven et al., 2006).

The effects of HBCDDs on immune parameters were investigated in the one-generation reproduction assay in Wistar rats which was enhanced for endocrine parameters (van der Ven et al., 2009). There was a statistically significant dose-response for decreased lymphocyte fraction, and a decreased whole white blood cell count in the blood and increased blood cell count in the bone marrow. Decreased thymus weight (both sexes) and increased poplietal lymph node weight (males) was seen, this was not accompanied by histopathological changes. The spleen marginal zone was enlarged in a higher frequency of top dosed animals than controls. In male offspring an increased IgG response after immunisation with sheep red blood cells (SRBC) was found ($BMDL_{20}$ 0.46 mg/kg b.w. per day) and also the fraction of neutrophilic granulocytes was increased ($BMDL_{20}$ 7.7 mg/kg b.w. per day).

Host immunity to respiratory syncytial virus (RSV) was investigated after exposure of five weeks old mice to 1 % HBCDDs (purity not stated) in the diet (approximately 1,700 mg/kg b.w. per day) for 28 days followed by RSV infection. No effect on the subsequent pulmonary viral titre was observed (Watanabe et al., 2010).

8.3.2.6. Liver

The few reports of hepatic effects describe changes in hepatic weight, gene expression or function.

Germer et al. (2006) measured changes in drug metabolism in the livers of juvenile male and female Wistar rats after 28 days of oral treatment. They found induction of CYP2B1/2B2 and CYP3A1/3A2, the latter in female rats only. In animals from the same study, van der Ven et al. (2006) reported increased absolute liver weights ($BMDL_{20}$ 22.9 mg/kg b.w. per day) and increased glucuronidation of T4 ($BMDL_{-10}$ 4.1 mg/kg b.w. per day) in females.

In a follow-up of the 28-days gavage study by van der Ven et al. (2006), Cantón et al. (2008) reported on changes in hepatic gene expression in HBCDD-treated juvenile rats. Female rats were found to exhibit more pronounced changes in gene expression than males. In particular, genes involved in cholesterol biosynthesis and lipid metabolism were down-regulated in females.

In the two-generation feeding study by Ema et al. (2008), increased absolute and relative liver weights were found at 101 mg/kg b.w. per day (male adults), 115 mg/kg b.w. per days (male weanlings), and 138 mg/kg b.w. per day (female weanlings), and in female adults at 1,142 mg/kg b.w. per day.

In the one-generation reproduction study by van der Ven et al. (2009), HBCDDs decreased the levels of apolar retinoids in female rat livers ($BMDL_{-10}$ 1.3 mg/kg b.w. per day).

In a lifetime bioassay (Kurokawa et al., 1984, as cited in ECB, 2008) B6C3F1 mice (50/sex/dose group) were exposed to HBCDD in the diet for 18 months at concentrations of 0, 100, 1,000 or 10,000 mg/kg in feed (equivalent to approximately 13, 130 or 1,300 mg/kg, respectively). This study is not reported according to current guideline, and only available as a study summary lacking significant details. According to ECHA (2008) the main changes in this test were liver lesions such as hepatocytic swelling; degeneration, necrosis, vacuole formation and fatty infiltration in the experimental groups in comparison with the control group. These changes are difficult to interpret due to lack of description of severity and absence of a clear dose-response relationship, but they support the observation that the liver is a target organ for HBCDDs.

In summary, HBCDDs increase liver weight and induces drug metabolism, mainly CYP2 and CYP3 and Phase II conjugating enzymes including thyroid hormone conjugating enzymes in the liver, particularly of female rats.

8.3.2.7. Genotoxicity

HBCDDs were not mutagenic in *Salmonella typhimurium* assay (Ames test) (Simmon et al., 1976; Zeiger et al., 1987; US-EPA, 1990).

In an *in vitro* mammalian cytogenetic test, using human peripheral blood lymphocytes, HBCDDs did not induce statistically significant increases in structural or numerical chromosomal aberrations (Gudi and Schadly, 1996).

Technical HBCDD tested in a concentration range of 0-20 µg/mL induced a slight, but statistically significant increase of somatic recombinations at the highest tested concentration in a non-standard assay using two clones of Chinese hamster V79 cell lines containing duplication mutations in the hprt gene (Helleday et al., 1999). In comparison with a clearly recombinogenic agent like Cr (VI), the effect was very small and the CONTAM Panel noted that the increase in recombination frequency was observed at a concentration that was clearly cytotoxic.

In an *in vivo* micronucleus assay, male NMRI mice were administrated HBCDDs intraperitoneally dissolved in dimethylsulfoxide (DMSO) at doses of 500, 1,000 or 2,000 mg/kg b.w., twice with a 24-hour interval between administrations. There was no significant dose-related increase in the frequency of micronuclei in the treated animals as compared to the vehicle controls (Engelhardt and Hoffman, 2000). At the highest dose there was evidence of a slight inhibition of erythropoiesis as reflected by the ratio of polychromatic to normochromatic erythrocytes.

Based on the available *in vitro* and *in vivo* studies it can be concluded that HBCDDs are not genotoxic.

8.3.2.8. Carcinogenicity

The carcinogenicity of HBCDDs has been evaluated in only one study, in mice. Kurokawa et al. (1984, as cited in ECB, 2008) exposed B6C3F1 mice (50/sex/dose group) to HBCDDs in their diet for 18 months, at concentrations of 0, 100, 1,000 or 10,000 mg/kg in feed. These concentrations of HBCDDs resulted in oral doses equivalent to approximately 13, 130 or 1,300 mg/kg b.w. per day, respectively. HBCDDs used in this study was described as a fine white powder, soluble in acetone and xylene, slightly soluble in benzene and olive oil and insoluble in water, but details of purity were not provided.

Survival at the end of the study was acceptable according to conventional guidelines for the interpretation of cancer bioassays, and did not differ amongst the groups. Body weight in the treated

groups was lower than in the controls, but this was thought by the study authors not to be related to HBCDDs administration. Changes were observed in the liver of male mice, but these showed little relationship to dose. The incidence of altered foci was increased in males at 130 mg/kg b.w. per day HBCDDs (41/45 versus 20/45 in controls) but not at 1,300 mg/kg b.w. per day (25/45). The incidence of carcinomas was increased in females, but did not show a dose-response relationship (males: 12/45, 21/47, 30/45, 24/45 in 0, 13, 120 and 1,300 mg/kg b.w. per day groups, respectively; females: 0/48, 0/49, 1/49, 5/49, respectively). The incidences of these liver tumours were within the background ranges in this strain of mouse as reported by Haseman et al. (1984).

This study was not reported in detail. However, in view of the weight of evidence that HBCDDs are not genotoxic and the essentially negative results of an 18-month study of carcinogenicity in mice, it is concluded that carcinogenicity is not a critical effect in the risk assessment of HBCDDs. ECHA (2009b) has concluded that the data available on carcinogenicity do not suggest a classification of HBCDD according to EU criteria.

8.3.3. Biochemical effects and molecular mechanisms

Experimental studies *in vivo* indicate that reproductive and neurodevelopmental toxicity, disturbance of thyroid homeostasis, hepatic hypertrophy and immunotoxicity are the major effects of HBCDDs. The majority of these effects appear to be a consequence either of activation of constitutive androstane receptor/pregnane X receptor (CAR/PXR) transcription, resulting in increased metabolism of thyroid and steroid hormones, or of modulation of target gene expression, due to interaction with nuclear hormone receptors, such as thyroid hormone receptors. Studies *in vitro* have provided evidence for these mechanisms and/or have revealed additional possible modes of action. However, it should be noted that many of the effects observed *in vitro* occur at concentrations 3-4 orders of magnitude above those normally found in the serum.

Zhang et al. (2008) reported the rank order of cytotoxicity of HBCDD stereoisomers and enantiomers in human hepatoma HepG2 cells. The rank order of stereoisomers is: β-HBCDD = γ-HBCDD > α-HBCDD. The (+)-HBCDD enantiomers were more potent than the (-)-HBCDD enantiomers. The rank order is (+)α-HBCDD = (+)γ-HBCDD > (+)β-HBCDD > (-)γ-HBCD followed by other two respective (-)-enantiomers. In general, potency was not very great, with concentrations of 10 μg/mL (~15 μM) producing 20-50 % loss in viability. There was some evidence that cytotoxicity in this model involved formation of reactive oxygen species (ROS), induction of nitric oxide synthase, dissipation of mitochondrial membrane potential and apoptosis (Zhang et al., 2008; Hu et al., 2009). The relevance of these studies to the *in vivo* effects of HBCDDs is unclear, but in general would appear to support the low acute toxicity of these compounds.

HBCDDs are agonists of human and rat PXR at μM concentrations, as shown *in vitro*, using reporter gene assays and determination of prototypical enzyme activities in liver cells (Fery et al., 2009, 2010). Studies *in vivo*, in rats, have shown that HBCDDs increase expression of a number of hepatic phase I and II biotransformation enzymes, including members of the CYP3A and CYP2B P450 sub-families, at the levels of mRNA, protein and enzyme activity, consistent with CAR/PXR activation (Germer et al., 2006; Cantón et al., 2008). In addition, evidence was obtained, at the mRNA levels for modulation of triacylglycerol and cholesterol metabolism (Cantón et al., 2008).

Importantly, no aryl hydrocarbon receptor (AhR)-mediated activity has been reported for HBCDDs (Behnisch et al., 2003; Hamers et al., 2006). α-HBCDD has been reported to suppress AhR mediated gene expression, with IC_{50} = 7.4 μM.

In vitro studies using reporter gene assays have shown that HBCDDs are androgen (α-, β- and γ-stereoisomers) and estrogen (β- and γ-stereoisomers) receptor antagonists, with IC_{50} values in the range 3.4-11.6 μM (Hamers et al., 2006). It is possible that such interactions contribute to the effects of HBCDDs observed on reproduction and bone mineral density. The CONTAM Panel noted that

hormones including estrogens, androgens, glucocorticoids and T4, affect the development of osteoclasts and osteoblasts by regulating the production and/or action of several cytokines (Manolagas, 2000). The observed decrease in bone mineral density may be also due to the decreased concentration of apolar retinoids in the liver (see Chapter 8.3.2.6). Retinoic acid may be the natural morphogen involved in vertebrate limb generation (Tabin, 1991) and the endogenous retinoids have been shown to be essential for chondrocyte maturation and endochondral ossification (Adams et al., 2007).

Both α- and β-HBCDD competed with T4 for binding to TTR transport protein *in vitro* (EC_{50} = 12 and 25 μM, respectively). All three stereoisomers (α-, β- and γ-HBCDD) potentiated T3/thyroid receptor-dependent cell proliferation in the rat pituitary cell line GH3, at micromolar concentrations (Hamers et al., 2006). Similar effects of HBCDDs were found in human cervical cancer (HeLa) cells transfected with the human TRα1 receptor (Yamada-Okabe et al., 2005).

Ibhazehiebo et al. (2011a, b) have reported that nanomolar concentrations of α-HBCDD suppress T3-induced TRα1- and TRβ1-mediated transcription and T3-induced neurite extension in granule cells and in cultured rat Purkinje cells. It was suggested that this was due to partial dissociation of the TRs from their specific DNA response elements. Disruption of neural differentiation in Purkinje cells is the most sensitive effect of HBCDDs reported *in vitro* to date.

HBCDDs have additional effects that may contribute to their neurotoxicity and neurodevelopmental effects. Technical HBCDDs can inhibit plasma membrane uptake of the neurotransmitters dopamine, glutamate and amino-n-butyric acid (IC_{50} = 4 μM) in rat synaptosomes (Mariussen and Fonnum, 2003). Exposure to low micromolar concentrations (LC_{50} 3 μM) of technical HBCDDs for 24h was cytotoxic to cerebellar granular cells (Reistad et al., 2006). Cell death was slightly reduced (by 23 %) by an NMDA receptor antagonist (MK801 3 μM), and 80 % by the antioxidant α-tocopherol (50 μM). In contrast, a calcium chelator (EGTA) or caspase 3 inhibitors had no effect on cell death. HBCDDs reduced the depolarization-induced increase in intracellular calcium concentration and neurotransmitter catecholamine release in rat pheochromocytoma PC12 cells, at concentrations of 2-20 μM (Dingemans et al., 2009).

Taken together, neurodevelopmental effects might be associated with modulation of thyroid hormone homeostasis and the involved processes include direct interaction of HBCDDs with thyroid hormone receptors, induction of CAR/PXR-dependent hormone-metabolizing enzymes, and/or perturbation of thyroid hormone transport.

The relative contribution of these effects to the neurotoxic and neurodevelopmental effects of HBCDDs remains to be determined.

Exposure of NK cells either for 1 h, followed by 24 h in HBCDD-free medium, or for 24h, decreased ATP levels and lytic function of the cells (Hinkson and Whalen, 2009). The reduced lytic effect appears to be due, at least in part, to decreased expression of cell-surface markers, such as CD16 and CD56, and cell binding function (Hinkson and Whalen, 2009).

The implications of these findings for the *in vivo* effects of HBCDDs on the immune system have yet to be determined.

8.4. Observations in humans

Only two studies were found in the literature regarding health effects on human exposure to HBCDDs.

Weiss et al. (2006) studied hydroxy-PCBs, PBDEs and HBCDDs in serum from an elderly population of Swedish fishermen and wives and association with bone mineral density. A previously established cohort of wives and ex-wives of professional fishermen from the Swedish east coast (Rylander et al.,

1995; Svensson et al., 1995) was approached for this study. A subset of 50 women born 1920-1954 was selected. Serum levels of α-HBCDD and γ-HBCDD were quantified by LC-MS/MS (median 0.5 ng/g fat) and bone mineral density (g/cm^2) was measured in the distal section of the forearm (radius and ulna) using dual energy X-ray absorptiometry (DXA). No associations were found between bone mineral density or biochemical markers of bone metabolism and HBCDDs. One caveat with this conclusion could be the limited number of samples, which hampered evaluation of weak or moderate associations.

To evaluate the association between HBCDDs and neonatal TSH, Eggesbø et al. (2011) studied HBCDDs measured in milk samples from 193 women who were part of the 'Norwegian Human Milk Study' (HUMIS), 2003-2006. TSH was measured in babies three days after delivery as part of the routine national screening program for early detection of congenital hypothyroidism. Additional information was obtained through the Medical Birth Registry and questionnaires to the mothers. They did not observe an association between HBCDDs in human milk and TSH in models adjusted for a number of possible confounders including other environmental toxicants.

8.5. Consideration of critical effects and possibilities for derivation of a health based guidance value

Toxicological studies with HBCDDs were performed with technical mixtures of which the purity and composition was not always specified. A typical composition for a mixture used in some of these studies was 9-13 % α-HBCDD, <0.5-12 % β-HBCDD and 72-90 % γ-HBCDD. The composition of these mixtures differs from the HBCDD profile found in wildlife and in foods, where α-HBCDD usually is the predominant stereoisomer.

The available toxicokinetics data, suggest that orally administered HBCDD is easily absorbed and rapidly distributed in different tissues, with some differences observed between γ- and α-isomer. In contrast to γ-HBCDD, α-HBCDD was found to concentrate in adipose tissue. Debromination and hydroxylation seem to be the major metabolic pathways for HBCDD, but stereoisomerisation of γ-isomer to α- and β-isomers was observed in mice treated with γ-HBCDD. Stereoisomerisation was not seen after exposure to α-HBCDD. Calculation of elimination half-lives of HBCDD stereoisomers in female mice, based on concentrations in adipose tissue, vary from 3-4 days for the γ-isomer, to 17 days for the α-isomer. The half-live was estimated to be 64 days (range 23-219 days) in humans for HBCDDs (sum of α-, β- and γ-HBCDD). The CONTAM Panel noted that this estimate was based on a rather high chronic dietary intake (142 ng/person per day) and that a lower intake would have resulted into longer half lives.

Toxicological studies have been carried out using different experimental designs with single or repeated dosing during gestation, postnatally or in adulthood using technical HBCDD. Main targets for HBCDD toxicity were the liver, thyroid hormone homeostasis, and the reproductive, the nervous and the immune systems.

Effects of technical HBCDD on the reproductive system were investigated in several studies in rats. In the two-generation reproduction toxicity study by Ema et al. (2008) the NOAEL for reduced fertility index and for the reduction of the number of ovarian primordial follicles is 10 mg/kg b.w. per day in F0 and F1 generations. In the one-generation reproduction study by van der Ven et al. (2009) the most sensitive effect on reproductive organs is a decrease in testes weight with a $BMDL_5$ of 11.5 mg/kg b.w. per day.

Animal studies with technical HBCDD provided evidence for disturbance of thyroid hormone homeostasis. In the 28-day study by van der Ven et al. (2006) the most sensitive effect was increased relative thyroid weight in females with a $BMDL_{10}$ of 1.6 mg/kg b.w. per day, although the CONTAM Panel had some concern about the reliability of this estimate, from visual inspection of the dose-response curve. The two-generation rat reproductive toxicity study (Ema et al., 2008) showed effects

on thyroid system with a LOEL of 100 mg/kg b.w. per day in F0 and F1 male animals, whereas the developmental study by Saegusa et al. (2009) indicated a NOEL of 100 mg/kg feed (corresponding to 8.1-21.3 mg/kg b.w. per day for maternal exposure), based on decrease in T3 and increase of relative thyroid weight in male offspring.

The CONTAM Panel noted that extrapolation of effects on thyroid hormone homeostasis observed in rodents to humans is complicated by differences in levels and binding capacity of transporting proteins, i.e. transthyretin and thyroid binding globulin (Capen, 1997; Hill et al., 1998), and feedback regulation of thyroid hormone homeostasis (Graham and Lake, 2008). In the case of HBCDDs, differences in changes in total serum T4 between rodents and humans may arise from the differences in activation of pregnane X receptor that leads to up-regulation of hepatic catabolic enzymes and a subsequent decline in circulating T4 concentrations (Schuetz et al., 1998).

In vitro reporter gene assays showed that HBCDDs are inducers of human and rodent PXR (Fery et al., 2009, 2010). Induction of metabolic enzymes observed by Germer et al. (2006) and Cantón et al. (2008) are also consistent with CAR/PXR activation. Competition of T4 with TTR transport protein and potentiation of T3-receptor dependent cell proliferation in a rat pituitary cell line (Hamers et al., 2006) represent additional mechanisms for changes in thyroid hormone homeostasis and signalling.

It is noted that thyroid hormone insufficiency in both humans and experimental animals may lead to neurodevelopmental effects (Miller et al., 2009). Therefore rodent data on the effects of HBCDDs on thyroid hormone levels or signalling might be of relevance for human health risk assessment.

Experimental studies in rodents have demonstrated that HBCDDs induced neurodevelopmental effects on behaviour. In a study with single administration Eriksson et al. (2006) exposed male NMRI mice to a dose of 0.9 or 13.5 mg/kg b.w. of technical HBCDD on PND10. Behavioural effects such as changes in rearing, locomotion and habituation, in response to a novel environment were already observed at 0.9 mg/kg b.w. Dose response analysis carried out by the CONTAM Panel resulted in a $BMDL_{10}$ of 0.93 mg/kg b.w. (see Appendix F). In a two-generation reproduction study by Ema et al. (2008), Crl:CD Sprague-Dawley rats showed significant neurodevelopmental effects in the F1 and F2 offspring at the highest dose (about 1,000 mg/kg b.w.) only. In addition, the absolute weight of the brain was decreased in adult rats and in F1 and F2 pups exposed to 15,000 mg/kg feed HBCDDs. Lilienthal et al. (2009) conducted a one-generation dietary study with Wistar rats, using a HBCDD mixture with dose levels ranging from 0.1 to 100 mg/kg b.w. per day. $BMDL_5$ values ranging from 0.6-4.4 mg/kg b.w. per day for reduced latencies to movement onset in catalepsy and of 0.2-0.9 mg/kg b.w. per day for increased thresholds in the brainstem auditory evoked potential (BAEP). In rat offspring from dams that were exposed from GD10 until PND10 effects on oligodendroglial development were observed at the highest dose (10,000 mg/kg feed equivalent to about 800-2,200 mg/kg b.w.) (Saegusa et al., 2009).

In the neurobehavioural study by Eriksson et al. (2006) using single administration on PND10, the litter effect was not taken into account appropriately since more than one pup per litter was allocated to a specific dose group tests. Since a difference in litter response may occur, uneven distribution of littermates over dose groups without statistical consideration of the litter as the experimental unit may bias the results of the analyses (Holson et al., 2008). Moreover, as studies on developmental effects on behaviour can be very variable (Crofton et al., 1991), it is important that when such an effect is to be used as the basis of a risk assessment, it should be independently verified.

In addition to the limitations and the concerns regarding the single administration protocol, the CONTAM Panel noted that there are also arguments supporting the use of the results from these studies. First, they provide the lowest doses leading to developmental effects on behaviour, and therefore need particular consideration. Second, due to the fact that effects are observed at PND10, these studies apparently cover a relevant neurodevelopmental period in experimental animals. Therefore, the CONTAM Panel concluded that in this specific case, the single-dose study should be considered for the assessments of risk to human health.

Neurodevelopmental effects were also reported after repeated administration, in which exposure is initiated prior to mating 10 weeks for males, 2 weeks for females, and continue through mating, gestation and lactation (Lilienthal et al., 2009). Of particular interests are the data showing sex-dependent effects on BAEP, with male offspring rats being more sensitive, and haloperidol induced catalepsy that was affected only in female offspring. While the sex-related differences in BAEP are difficult to explain, the reduced latency to movement onset in the haloperidol induced catalepsy may be due to the higher induction of P450 enzymes in female offspring, which can affect haloperidol degradation.

Effects on the immune system were revealed in two studies. In a 28 day study with rats (van der Ven et al., 2006) reduced splenocyte counts were found with a $BMDL_{20}$ of 104 mg/kg b.w. per day. In a one-generation reproduction study (van der Ven et al., 2009) an increased IgG response after immunisation with SRBC was found in male offspring with a $BMDL_{20}$ of 0.46 mg/kg b.w. per day. Also the fraction of neutrophilic granulocytes was increased ($BMDL_{20}$ 7.7 mg/kg b.w. per day). The CONTAM Panel noted however that for both effects the ratio between the BMDL and the benchmark dose upper limit (BMDU) was rather large (>10) indicating a large variation in the dose-response data. Due to the uncertainty in the data the CONTAM Panel concluded not to use these BMDLs as reference point for the hazard characterisation.

The liver is a target organ for effects of HBCDDs. The most prominent hepatic effects are induction of metabolising enzymes (Germer et al., 2006), including T4 glucuronidation ($BMDL_{10}$ 4.1 mg/kg b.w. per day), and liver enlargement ($BMDL_{20}$ 22.9 mg/kg b.w. per day) observed in the 28-days study by van der Ven et al. (2006). Changes in hepatic drug metabolism and transthyretin expression seem to play a key role in the decrease in serum T4 observed in rodents. In addition, HBCDDs can lead to a decrease in apolar hepatic retinoids in the liver of female rats ($BMDL_{10}$ 1.3 mg/kg b.w. per day). The CONTAM Panel noted that for this effect the ratio between BMDL and BMDU is >10-fold, indicating a large variation in the dose-response data. Due to the uncertainty in the data the Panel concluded not to use this BMDL as reference point for the hazard characterisation.

The CONTAM Panel noted that effects on bone mineral density were observed with a $BMDL_{10}$ of 0.056 mg/kg b.w. per day (van der Ven et al., 2009). The observed effect might be due to the interaction of HBCDDs with steroid hormone system, and/or decrease in retinoids. The Panel noted that the ratio between the $BMDL_{10}$ and the $BMDU_{10}$ for effects on bone mineral density was very large (about a factor of 20) indicating a large variation in the dose-response data. Due to the uncertainty in the data the Panel concluded not to use this BMDL as reference point for the hazard characterisation, and future observations need to confirm this effect.

There is no evidence for a teratogenic potential of HBCDDs.

The available studies indicate that HBCDDs are not genotoxic.

There is limited information from only one long-term toxicity/carcinogenicity study for HBCDDs in B6C3F1 mice (Kurokawa et al., 1984, as cited in ECB, 2008). The incidence of altered foci in the liver of males was increased, as was the incidence of liver carcinoma in females, but without a dose-relationship. In addition, the CONTAM Panel noted that the incidence of liver carcinoma was within the range of background levels for this strain of mice. Given the lack of genotoxicity the Panel concluded that the carcinogenicity is not a critical effect in the hazard characterisation of HBCDDs. Also ECHA (2009b) has concluded that the data available on carcinogenicity do not suggest a classification of HBCDD according to EU criteria.

Only two epidemiological studies were available on the health effects of human exposure to HBCDDs. Weiss et al. (2006) were not able to establish an association between the levels of HBCDDs in blood and bone mineral density in an elderly female population. One caveat, however, could be the limited number of samples, which hampered evaluation of weak or moderate associations. Eggesbø et al. (2011) studied the association between levels of HBCDDs in human milk and neonatal thyroid-

stimulating hormone (TSH). No association was found adjusting for a number of confounders, including environmental toxicants.

Since all toxicity studies were carried out with technical HBCDD, a risk assessment of individual stereoisomers was not possible. The CONTAM Panel concluded that the most critical effects for technical HBCDD were effects on thyroid weight in female rats from an 28-days study with a $BMDL_{10}$ of 1.6 mg/kg b.w. per day (van der Ven et al., 2006), although the CONTAM Panel had some reservations about the numerical estimate of this value, and behavioural effects in male mice from the study with single administration (Eriksson et al., 2006) with a $BMDL_{10}$ of 0.93 mg/kg b.w. The CONTAM Panel decided to use the $BMDL_{10}$ of 0.93 mg/kg b.w. as reference point for the hazard characterisation. A benchmark response (BMR) of 10 % was chosen to avoid extrapolation beyond the observable range (EFSA, 2009).

In Chapter 8.1.4.2, slower elimination kinetics of HBCDDs in humans compared to rodents have been indicated. As a consequence, exposure to similar external doses of HBCDDs will result in higher concentrations in the human body than in the rodent. Therefore, the use of external dose levels of HBCDDs associated with toxic effects in animals is not appropriate for the risk assessment in humans. Instead, the body burden provides a more appropriate dose metric for a direct comparison of internal effect doses in animals and in humans. Body burdens corresponding with effects of HBCDDs in rodents, even resulting from studies with different dose regimens (e.g. single vs. repeated administration), can readily be transformed (see Chapter 9) into estimated human daily intakes that on a chronic basis would be expected to lead to similar body burdens in humans under steady state conditions.

For the $BMDL_{10}$ of 0.93 mg/kg b.w. for effects on behavior in mice after single oral administration and assuming an absorbed fraction of 0.85 the corresponding body burden would be about 0.79 mg/kg b.w. The CONTAM Panel noted that studies with repeated administration would have resulted in considerably higher body burdens.

The body burden of 0.79 mg/kg b.w. could in principle be used as the basis to derive a human health based guidance value, e.g. a tolerable daily intake. The CONTAM Panel concluded, however, that due to the limitations and uncertainties in the current data base on HBCDDs, the derivation of a health based guidance value was not appropriate. Instead, the Panel used a margin of exposure[17] (MOE) approach for the risk characterisation of HBCDDs.

9. Risk characterisation

In general, food is the main contributor to human exposure to HBCDDs. Therefore, the chronic dietary intake ($D_{r,h}$) which corresponds to a given body burden, can be calculated. In general this calculation needs kinetic modeling, but since HBCDDs are expected to distribute predominantly in the adipose tissue in humans, a one compartmental model will suffice here (for clarification see Appendix E). To calculate the chronic human dietary intake ($D_{r,h}$) which is associated with the steady state body burden at the BMDL, only two additional parameters are needed, the fraction of the daily intake which is absorbed in the body and the rate constant for the elimination of the compounds from the body. Therefore $D_{r,h}$ can be calculated as follows:

[17] The margin of exposure (MOE) is the ratio between a defined point on the dose-response curve for the adverse effect and the human intake.

$$D_{r,h} = \frac{BB_a \cdot k_{el,h}}{F_{abs,h}} \quad (1)$$

$k_{el,h}$ = rate constant for the elimination from the human body (day^{-1})
$F_{abs,h}$ = fraction of the chemical in food which is absorbed into the human body (dimensionless)
$D_{r,h}$ = chronic human daily intake (amount/kg b.w. per day)
BB_a = body burden in the experimental animal (amount/kg b.w.)

It should be noted that in a one-compartment model as applied in this case, the relationship holds that $k_{el} = \ln 2/t_{1/2}$ (with $t_{1/2}$ being the elimination half-life in the body, i.e. the time needed for half of the amount of a chemical to be cleared from the body once the exposure has stopped).

9.1. Margin of exposure (MOE)

By comparison of the calculated human dietary intake associated with the body burden at the BMDL ($D_{r,h}$) with the estimated dietary intake provided in Chapter 7.4 for the general population or for specific subgroups of the population, the margin of exposure (MOE) can be calculated, where MOE = $D_{r,h}$ (amount/kg b.w.) /estimated dietary intake (amount/kg b.w.).

The CONTAM Panel estimated the MOE for different age classes of adult consumers, children (3-9 years) and adolescents (10-17 years) with an average and high consumption. Also specific subgroups of the general population such as high and frequent fish consumers, and people with a high consumption of fish liver were considered. Dietary intake of infants (< 1 year) and toddlers (1-3 years) was not considered because the Panel was of the opinion that the available data were too limited to facilitate a reliable assessment of the exposure. However, the MOE for breast-fed infants could be estimated.

As indicated in Chapter 8.5, the body burden at the $BMDL_{10}$ is 0.79 mg/kg b.w. As a 'worst-case' the longest human half-life identified for HBCDDs (see Chapter 8.1.4.2) of 219 days was used. In the absence of robust information, the human absorption of HBCDDs is assumed to be 100 % ($F_{abs,h}$ = 1). Substituting these figures into formula (1) leads to an estimated chronic human dietary intake ($D_{r,h}$) of 0.003 mg/kg b.w. per day associated with the body burden at the $BMDL_{10}$ for HBCDDs.

For average adult consumers the minimum LB and maximum UB dietary intake across European countries is 0.09 and 0.99 ng/kg b.w. per day, respectively. This provides a MOE of about 30,000 and 3,000, respectively, when compared with the estimated chronic human dietary intake ($D_{r,h}$) of HBCDDs of 0.003 mg/kg b.w. per day associated with the body burden at that $BMDL_{10}$. For high adult consumers (P95) the minimum LB and maximum UB dietary intake across European countries is 0.39 and 2.07 ng/kg b.w. per day, respectively. This provides MOEs of about 7,700 and 1,450. The 'elderly' and 'very elderly' population has a lower HBCDD intake than the age group of adults and thus higher MOEs.

The specific subgroup high and frequent fish consumers had a minimum LB and maximum UB dietary intake across European countries of 1.34 and 2.76 ng/kg b.w. per day, respectively. This results in MOEs of about 2,200 and 1,100. For consumers of fish liver a minimum LB and maximum UB dietary intake of 1.03 and 1.94 ng/kg b.w. per day, respectively, was estimated. This intake results in MOEs of about 3,000 and 1,500.

For children of the age of 3-10 years with an average consumption the minimum LB and maximum UB dietary intakes are 0.15 and 1.85 ng/kg b.w. per day, respectively. These result in MOEs of 20,000 and 1,600, respectively. When they had a high consumption (P95) the minimum LB and maximum UB

dietary intake was 0.8 and 4.46 ng/kg b.w. per day, respectively. The resulting MOEs are about 3,700 and 700, respectively.

Usually a MOE of 100, covering uncertainties and variability with respect to kinetic and dynamic differences between animal species and humans (factor 4 × 2.5 = 10) and within the human population (factor 3.2 × 3.2 = 10), is considered sufficient to conclude that there is no health concern. Since the MOE approach is based on a body burden comparison between animals and humans, the potential kinetic differences between animals and humans have been accounted for. Equally, by focussing on the body burden associated with a $BMDL_{10}$ for neurobehavioural effects in mice induced during a relevant period for brain development, and applying this body burden to the entire life span in humans, individual difference in susceptibility has been covered. Therefore, the calculated MOE should be sufficient to cover inter-species differences in dynamics for the effects observed (factor 2.5). Considering the uncertainty in the elimination half-life in humans, the CONTAM Panel concluded that the MOE should also cover individual differences in kinetics (factor 3.2). This implies that a MOE larger than 8 (2.5 × 3.2) might indicate that there is no health concern.

All the MOEs reported above are much higher than a factor of 8. Also considering the large difference between minimum LB and maximum UB intake estimates, the CONTAM Panel concluded that current dietary intake to HBCDDs does not raise a health concern.

For breast-fed infants with average human milk consumption (800 mL per day) the reported range for total HBCDD in human milk (0.13-31 ng/g fat) results in daily exposures of 0.60-142 ng/kg b.w. This results in a MOE ranging from 5,000 to 21. For infants with high human milk consumption (1,200 mL per day) the intake is 0.90-213 ng/kg b.w., resulting in a MOE ranging from 3,300 to 14. The lowest MOEs for breast-fed infants with average and high consumption are about twice the value of 8 mentioned above. Considering that consumption of human milk occurs only through a restricted period in life the CONTAM Panel concluded that it is unlikely that exposure via human milk raises a health concern.

Due to lack of data the CONTAM Panel was not able to assess the dietary intake of infants (< 1 year) and toddlers (1-3 years) but assumed that dietary intake to contaminants such as HBCDD of these age groups usually is lower than that of breast-fed infants. Therefore it concluded that it is also unlikely that dietary exposure to HBCDDs of infants and toddlers will raise a health concern.

Dust in homes, classrooms and cars, can be an additional source of exposure to HBCDDs for children. Based on a 'typical' exposure scenario (see Chapter 7.6) exposure of young children (1-6 years of age) to HBCDDs through ingestion of dust has been estimated to be about 5.9 ng/kg b.w. per day. In the same study a 'high' exposure scenario resulted in an estimated daily exposure through dust of 330 ng HBCDDs/kg b.w. The CONTAM Panel noted however that considerably lower intake estimates for HBCDDS from dust has been reported in another study. It therefore concluded that the 'typical' exposure scenario provided the most realistic estimate of exposure to HBCDDs from dust. This 'typical' exposure estimate results in an MOE of about 500. Combining the UBs of average or high dietary intake of children of this age group with this 'typical' dust exposure leads to a total exposure to HBCDDs of about 7.7 and 10.3 ng/kg b.w., respectively. The resulting MOEs are about 390 and 300, respectively. Taking into account the uncertainties in the dust exposure estimates and considering the use of UB intake estimates, the CONTAM Panel concluded that the available information indicates that it is unlikely that additional exposure to HBCDDs from dust raises a health concern.

9.2. Comparison of body burdens

Since the half-life of HBCDDs in humans have not been directly measured, but estimated based on the assumption of a steady state dietary intake, and the concerns regarding the intake value used, the CONTAM Panel also considered information on biomarkers of exposure to assess the health risk of exposure to HBCDDs. This was done for comparison with the results of the MOE approach as

presented above. In Chapter 8.2, information on levels of individual HBCDDs in human adipose tissue and serum has been presented. The CONTAM Panel identified information on concentrations in adipose tissue as being most relevant, because they best reflect long-term exposure to HBCDDs. In order to facilitate a direct comparison of the actual human body burdens with the body burden at the $BMDL_{10}$ the CONTAM Panel converted reported concentrations in adipose tissue in humans to an overall body burden by assuming an average fat content of the human female adult body of 25 % (van der Molen, 1998).

Concentrations of total HBCDD in individual samples of adipose tissue range from <0.5 to 7.5 ng/g fat. When these values are converted into body burden concentrations assuming a fat content of 25 % for the human female adult body, and these body burdens are compared with the body burden at the $BMDL_{10}$ of 0.79 mg/kg b.w., a margin of about 420 to >6,300 can be estimated. Since the information on total HBCDD in adipose tissue is limited to only one study, the CONTAM Panel also compared the body burdens based on concentrations of total HBCDD in serum from non-occupationally exposed European populations. These concentrations (individual samples) ranged from <0.2 to 7.4 ng/g fat. Converting these concentrations into body burdens results in a margin of about 425 to >15,800. The CONTAM Panel concluded that this result supports the conclusion that current dietary exposure to HBCDDs in the EU does not raise a health concern.

10. Uncertainty

The evaluation of the inherent uncertainties in the assessment of exposure to HBCDDs has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2007). In addition, the report on 'Characterizing and Communicating Uncertainty in Exposure Assessment' (WHO/IPCS, 2008) has been considered. According to the guidance provided by the EFSA opinion (EFSA, 2007) the following sources of uncertainties have been considered: assessment objectives, exposure scenario, exposure model, and model input (parameters).

10.1. Assessment objectives

The objectives of the assessment were clearly specified in the terms of reference. The CONTAM Panel assessed the new occurrence data that were collected by EFSA, and carried out a dietary exposure assessment for the general population as well as for specific subgroups. The uncertainty in the assessment objectives is considered to be negligible.

10.2. Exposure scenarios/Exposure model

In response to EFSA's request to submit HBCDD occurrence data in food, seven European countries submitted data on 1,914 samples collected and analysed between 2000 and 2010. Norway provided 47 % of the data followed by France (14 %), UK (14 %) and Sweden (13 %).

The food products for which data were provided varied between submissions from the different European countries, but most samples belonged to the food group of 'Fish and other seafood', followed by 'Meat and meat products', 'Eggs and egg products' and 'Milk and dairy products' and only a few samples of plant origin. Thus, the dietary exposure assessment was only based on a limited number of food groups. Occurrence data were submitted either stereoisomer-specific or as total HBCDD not differentiating between the different stereoisomers. Chronic dietary exposure is based on total HBCDD in the food group of 'Fish and other seafood', as well as the sum of the three stereoisomers for the food groups 'Meat and meat products', 'Eggs and egg products' and 'Milk and dairy products'. The use of the latter may lead to some overestimation because of the inclusion of non detected stereoisomers in the calculation of the UB sum. There is uncertainty with respect to regional differences in HBCDD contamination of food commodities, and the CONTAM Panel recognised that

the data set is not representative of food on the EU market. Reports on the effects of food processing on the HBCDD levels in prepared food commodities are scarce. However, it can be assumed that HBCDDs behave similarly to other brominated lipophilic contaminants, such as PBBs. Taking into account that the processed foods in general had lower PBB concentrations than the respective raw materials, this could have led in a similar way to some overestimation of the overall HBCDD dietary exposure.

The high proportion of samples having levels below the LOD or LOQ introduced uncertainties to the overall estimate. Because of the high proportion of samples below the LOD or LOQ, all the dietary exposure calculations were based on the mean lower bound and upper bound concentrations. The use of the upper bound in this opinion tends to overestimate the dietary exposure. It is generally accepted that the use of the mean contamination to represent the long term dietary exposure is expected to be an overestimation compared with the use of the median. Taken together, the uncertainties regarding the exposure estimates are considered to overestimate the dietary exposure.

The occurrence data for HBCDDs reported for human milk in a study carried out in A Coruña showed very high levels and an unusual deviant stereoisomer pattern from other human specimens with γ-HBCDD in most samples dominating over α-HBCDD. As this points to a specific so far unknown contamination source, the respective data were excluded from the risk assessment. An inclusion of these data would add a considerable uncertainty to the overall risk assessment.

The CONTAM Panel noted that information on non-dietary exposure (e.g. dust) is scarce. This adds to the uncertainty of total exposure (diet and dust) to HBCDD, particularly for children.

10.3. Model input (parameters)

There are no prescribed fixed official methods for the analysis of HBCDDs and laboratories can use any method of analysis, provided it can be demonstrated in a traceable manner that it fulfils the requirements according to ISO 17025. This may have added to the uncertainty in the analytical results. The scarce number of certified reference materials is a limitation when the method performance for the analytical methods for analysis of HBCDDs in food is assessed, and adds thereby to the overall uncertainty in the analytical results.

10.4. Other uncertainties

Most of the toxicological data were gained from experiments with technical HBCDD rather than purified individual stereoisomers. Moreover, the stereoisomer profile found in technical HBCDD does not resemble the profiles found in food and humans. Whether the different profiles are due to conversion of the stereoisomers or due to different bioaccumulation behaviour is currently not known. Limited data are available on the toxicokinetics of HBCDDs, particularly in humans. As the toxicity of the individual stereoisomers is not known, and the toxicological reference point is based on information from technical HBCDD, this adds a considerable uncertainty to the risk assessment.

The relevance for human risk assessment of some of the end-points obtained in experimental studies is not clear, particularly for neurodevelopmental effects, thyroid effects and gender-related differences.

The CONTAM Panel noted that litter effects were not taken into account appropriately in the study with single administration of HBCDDs. Uneven distribution of littermates over dose groups without statistical consideration of the litter as the experimental unit may have biased the results of the study. This provides an additional source of uncertainty.

10.5. Summary of uncertainties

In Table 18 a summary of the uncertainty evaluation is presented, highlighting the main sources of uncertainty and indicating an estimate whether the respective source of uncertainty might have led to and over- or underestimation of the dietary exposure or the resulting risk.

Table 18: Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the dietary exposure to HBCDDs.

Sources of uncertainty	Direction
Measurement uncertainty of analytical results	+/- [(a)]
Limited number of food groups analysed	+/-
Extrapolation of occurrence data from a few countries to whole Europe	+/-
Different way of reporting occurrence data (stereoisomer-specific or total HBCDD)	+/-
Influence of upper-bounds for non-detects on dietary exposure estimate	+
Lack of information on the impact of food processing	+/-
Information on other sources of exposure (e.g. dust) is limited	-
Limited information on the purity of technical HBCDDs used in animal studies.	+
Isomeric profiles of technical HBCDDs used in toxicological studies do not resemble the profiles found in food	+/-
Limited data on the toxicokinetics of HBCDDs, particularly in humans.	+/-
Lack of information on the toxicity of the individual stereoisomers	+/-
Relevance of the toxicological effects to humans	+

(a): + = uncertainty with potential to cause over-estimation of exposure/risk; - = uncertainty with potential to cause under-estimation of exposure/risk.

The CONTAM Panel considered that the impact of the uncertainties on the risk assessment of dietary exposure to HBCDDs is considerable and concluded that its assessment of the risk is likely to be conservative – i.e. more likely to overestimate than to underestimate the risk.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

General

- Hexabromocyclododecanes (HBCDDs) are stereoisomers produced by bromination of cyclododecatriene. The three main stereoisomers are α-HBCDD, β-HBCDD and γ-HBCDD. Each of the stereoisomers forms a pair of enantiomers.
- γ-HBCDD is the major constituent of technical HBCDD.
- Technical HBCDD is widely used as an additive flame retardant, and therefore HBCDDs can leach into the environment. Technical HBCDD is primarily used in expanded and extruded polystyrene applied as construction and packing materials, and also used in textiles.
- The chemical stability of the HBCDD stereoisomers varies, with α-HBCDD being most stable.
- HBCDD stereoisomers are susceptible to elimination of HBr, reductive debromination and radical reactions. Abiotic transformation of γ-HBCDD to α-HBCDD has been shown.

- α-HBCDD has the highest bioaccumulative potency among the stereoisomers in technical HBCDD.

- HBCDDs are found as wide-spread contaminants in the global environment especially in top predators.

Occurrence

- Following a European Food Safety Authority (EFSA) call for data, analytical results from 1,914 food samples were provided to EFSA by seven European countries, covering the period from 2000 to 2010.

- The present data are divided into total HBCDD as mostly determined by gas chromatography-mass spectrometry (GC-MS), and stereoisomer-specific data of α-HBCDD, β-HBCDD and γ-HBCDD as mainly analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

- The data were characterised by a high proportion of non-detects, therefore only those food groups where the sample size was greater than 50 observations (or there were more than 25 positive samples), and when the percentage of non-detects was less than 80 % were used for the total dietary exposure assessment, i.e. 'Fish and other seafood', 'Meat and meat products', 'Milk and dairy products' and 'Eggs and egg products'.

- 'Fish and other seafood' is the food group with the highest number of samples, including fish meat, water molluscs, fish liver, crustaceous, fish roe and fish products.

- Overall α-HBCDD is the main contributor to the total levels of HBCDDs in all food groups with the lower proportion of non-detects.

- Investigations on HBCDD levels in human milk are scarce. Most of the data are not stereoisomer-specific, but given as total HBCDD. In those samples that were analysed stereoisomer-specific, generally α-HBCDD predominates with levels between 0.75 and 19.71 ng/g fat.

- Levels of total HBCDD in human milk ranged from 0.13 to 31 ng/g fat. Considerably higher levels were found in one study where the sum for the three main HBCDD stereoisomers ranged from 3 to 188 ng/g fat with most samples showing higher levels for γ-HBCDD than α-HBCDD.

- The median concentration of total HBCDD in serum and adipose tissue samples was in general not higher that 3 ng/g fat. α-HBCDD was found to be the dominating stereoisomer. When considering occupational exposure the levels were reported to be up to 101 ng/g fat and a much higher contribution of γ-HBCDD was reported.

Human exposure

- Chronic dietary exposure assessment is based on the concentration of total HBCDD in the food group of 'Fish and other seafood' and the sum of the individual HBCDD stereoisomers (α-, β- and γ-HBCCD) for 'Eggs and egg products', 'Milk and dairy products' and 'Meat and meat products'.

- The highest mean estimated dietary exposure to HBCDDs across the European dietary surveys is for children from three to ten years old ('Other children') and is between 0.15 to 1.85 ng/kg body weight (b.w.) per day (minimum lower bound (LB) and maximum upper bound (UB),

respectively). Total dietary exposure for adults is around half the exposure for 'Other children', with minimum LB and maximum UB of respectively 0.09 and 0.99 ng/kg b.w. per day.

- Due to the few occurrence data for 'Food for infants and small children' and the restricted number of consumption data for infants, the Panel on Contaminants in the Food Chain (CONTAM Panel) decided that it was not appropriate to assess dietary exposure to HBCDDs for infants and toddlers on the basis of the data available.

- For high consumers (95th percentiles), the dietary intake of HBCDDs across European countries for 'other children' are between 0.80 and 4.46 ng/kg b.w. per day (minimum LB and maximum UB, respectively). The corresponding data for 'adults' are between 0.39 and 2.07 ng/kg b.w. per day, respectively.

- For high fish consumers the mean dietary exposure to HBCDDs ranges from 1.34 to 2.76 ng/kg b.w. per day (minimum LB and maximum UB).

- Fish liver, a traditional food in certain regions of Europe, was identified as an important source of dietary exposure to HBCDDs for consumers. The mean dietary exposure to HBCDDs for consumers of fish liver once per week is between 1.03 and 1.94 ng/kg b.w. per day (minimum LB and maximum UB, respectively).

- As contamination of food samples of plant origin is generally lower than that of food samples of animal origin, it can be assumed that the dietary exposure to HBCDDs for vegetarians is lower than that for people consuming a mixed diet.

- The exposure scenario based on average consumption of human milk (800 mL per day) and the reported range for total HBCDD results in daily exposures of about 0.6-142 ng/kg b.w. For infants with high human milk consumption (1,200 mL per day) the respective daily exposures range from 0.90 to 213 ng/kg b.w.

- Non-dietary exposure, mainly through dust in homes, offices, schools, cars and public environment can substantially contribute, and in some cases even dominate the total human exposure to HBCDDs, especially for toddlers and other children.

Hazard identification and characterisation

- The acute toxicity of HBCDDs is low.

- In rodents, HBCDD stereoisomers are almost completely absorbed through the gastrointestinal tract, and residues are mainly distributed to adrenals, liver, skin and muscle (γ-HBCDD), or to adipose tissues, liver, skin and muscle (α-HBCDD).

- In repeated dose experiments α-HBCDD, but not γ-HBCDD, accumulates mainly in adipose tissue.

- Metabolic debromination and hydroxylation of HBCDDs, and conversion of γ-HBCDD to α- and β-HBCDD have been reported.

- The elimination half-lives of HBCDD stereoisomers in female mice, based on concentrations in adipose tissue, vary from 3-4 days for γ-HBCDD, to 17 days for α-HBCDD. In humans the half-life was estimated to be 64 days (range 23-219 days) for the sum of α-, β- and γ-HBCDD stereoisomers.

- Toxicological studies have been carried out using different experimental designs with single or repeated administration during gestation, postnatally or in adulthood using technical HBCDD. Main targets for toxicity were the liver, thyroid hormone homeostasis, the reproductive, the nervous and the immune systems.

- The developmental studies did not demonstrate teratogenicity or fetotoxicity of technical HBCDD in rats. However in the two-generation reproduction study an increased pup mortality during lactation was observed.

- Exposure to technical HBCDD increases liver weight and induces drug metabolism, mainly CYP2 and CYP3 and Phase II conjugating enzymes in the liver of rodents. It leads to an increase in hepatic glucuronidation capacity for thyroxine (T4) in female rat liver.

- Exposure to technical HBCDD during development in rodents affects the nervous system with subsequent behavioural changes. Alterations in the thyroid hormone regulation may play a critical role in the onset of the observed effects. Effects on the immune system (increased immunoglobulin G response) were also observed.

- Mechanistic studies indicate that perturbation of thyroid hormone receptors and consequent suppression of neural cell differentiation, induction of constitutive androstane receptor (CAR)/pregnane X receptor (PXR)-dependent thyroid hormone metabolizing enzymes and/or modulation of thyroid hormone binding to transport proteins, might contribute to the neurodevelopmental adverse effects of HBCDDs.

- Technical HBCDD is not genotoxic.

- Given the negative results of an 18-month study of carcinogenicity in mice and the fact that HBCDDs are not genotoxic, it is concluded that carcinogenicity is not a critical effect.

- In epidemiological studies, no association was found between the levels of HBCDDs in blood and bone mineral density in an elderly female population, and no association between HBCDDs in human milk samples and thyroid-stimulating hormone (TSH) in neonates.

- Based on neurodevelopmental effects on behaviour in mice the CONTAM Panel derived a $BMDL_{10}$ value (the lower confidence limit for the benchmark dose of a 10 % change) for HBCDDs of 0.93 mg/kg b.w.

- Because the elimination kinetics for HBCDDs between experimental animals and humans differ, the CONTAM Panel converted the $BMDL_{10}$ into an estimated chronic human dietary intake associated with the body burden at the $BMDL_{10}$, as basis for the risk assessment. The estimated human dietary daily intake associated with the body burden at the $BMDL_{10}$ is 0.79 mg/kg b.w.

- The CONTAM Panel concluded that due to the limitations and uncertainties in the database the derivation of health based guidance values for HBCDDs was not appropriate. Instead, a margin of exposure (MOE) approach was used for the risk characterization.

Risk characterisation

- The MOEs between the intake associated with the body burden at the $BMDL_{10}$ and the estimated dietary intake for the different population groups indicate that current dietary exposure to HBCDDs in the European Union does not raise a health concern.

- It is unlikely that exposure of breast-fed infants via human milk raises a health concern.

- Additional exposure to HBCDDs from dust, particular for children, is unlikely to raise a health concern.

RECOMMENDATIONS

- Surveillance of HBCDDs should continue and include stereoisomer specific information, since technical HBCDD is still produced and present in numerous products in use.
- Occurrence data in food groups relevant for exposure of infants and toddlers would be of value to refine the exposure assessment.
- Monitoring of levels of HBCDD stereoisomers in humans, e.g. human milk samples, should continue.
- Any further toxicological studies of HBCDDs should be conducted with pure and characterised individual HBCDD stereoisomers most relevant to human exposure, and carried out according to appropriate and relevant study designs for risk characterisation. Such studies should also include investigations of the mechanisms involved.
- Epidemiological studies of HBCDDs are required with suitable estimates of human exposures.

REFERENCES

AFSSA (Agence Française de Sécurité Sanitaire des Aliments), 2009. Report of the 2006/2007 Individual and National Study on Food Consumption 2 (INCA 2). Synthèse de l'étude individuelle nationale des consommations alimentaires 2 (INCA 2), 2006-2007. 1-44.

Abdallah MA-E, Harrad S, Ibarra C, Robson M and Covaci A, 2008a. Hexabromocyclododecanes in indoor dust from Canada, the United Kingdom and the United States. Environmental Science and Technology, 42, 459-464.

Abdallah MA-E, Harrad S and Covaci A, 2008b. Hexabromocyclododecanes and tetrabromobisphenol-A in indoor air and dust in Birmingham, UK: Implications for human exposure. Environmental Science and Technology, 42, 6855-6861.

Abdallah MAE, Ibarra C, Neels H, Harrad S and Covaci A, 2008c. Comparative evaluation of liquid chromatography-mass spectrometry versus gas chromatography-mass spectrometry for the determination of hexabromocyclododecanes and their degradation products in indoor dust. Journal of Chromatography A, 1190, 333-341.

Abdallah MAE and Harrad S, 2011. Tetrabromobisphenol-A, hexabromocyclododecane and its degradation products in UK human milk: relationship to external exposure. Environment International, 37, 443-448.

Abdallah MA-E and Harrad S, 2009. Personal exposure to HBCDs and its degradation products via ingestion of indoor dust. Environment International, 35, 870-876.

Adams SL, Cohen AJ, Lassova L, 2007. Integration of signaling pathways regulating chondrocyte differentiation during endochondral bone formation. Journal of Cellular Physiology, 213, 635-641.

Albemarle Corporation, 2000. Saytex 9006L Flame Retardant. Baton Rouge. Lousiana: Albemarle Corporation. 2 pp.

Allchin CR and Morris S, 2003. Hexabromocyclododecane (HBCD) diastereoisomers and brominated diphenyl ether congener (BDE) residues in edible fish from the rivers Skerne and Tees, U.K. Organohalogen Compounds, 61, 41-44.

Antignac JR, Cariou R, Maume D, Marchand P, Monteau F, Zalko D, Berrebi A, Cravedi JP, Andre F and Le Bizec B, 2008. Exposure assessment of fetus and newborn to brominated flame retardants in France: preliminary data. Molecular Nutrition and Food Research, 52, 258-265.

Arsenault G, Konstantinov A, Marvin CH, Macinnis G, McAlees A, McCrindle R, Riddell N, Tomy GT and Yeo B, 2007. Synthesis of the two minor isomers, delta- and epsilon-1,2,5,6,9,10-hexabromocyclododecane, present in commercial hexabromocyclododecane. Chemosphere, 68, 887-892.

Asplund L, Bugnert A and Nylund K, 2004. Comparison of spatial and temporal trends of methoxylated PBDEs, PBDEs and hexabromocyclododecane in herring along the Swedish coast. Organohalogen Compounds, 66, 3988-3993.

Barontini F, Cozzani V, Cuzzola A and Petarca L, 2001. Investigation of hexabromocyclododecane thermal degradation pathways by gas chromatography/mass spectrometry. Rapid communications in Mass Spectrometry, 15, 690-698.

Becher G, 2005. The stereochemistry of 1,2,5,6,9,10-hexabromocyclododecane and its graphic representation. Chemosphere, 58, 989-991.

Behnisch PA, Hosoe K and Sakai S, 2003. Brominated dioxin-like compounds: *in vitro* assessment in comparison to classical dioxin-like compounds and other polyaromatic compounds. Environment International, 29, 861-877.

Bergman Å, Hovander L, Sundström M, Athanassiadis I, Athanasiadou M, Sällsten G, Bignert A and Nyberg E, 2010. Collection and chemical analysis of environmental contaminants in Swedish mother's milk – Results from 2008-2010 (in Swedish). Swedish EPA Report # 215 0707/215 0709, 1-22.

Bergsten C, 2004. Fish- and game study, Part B. Norwegian Food Safety Authority. Available from http://www.mattilsynet.no/english/___/fish_and_game_study_9800

Bokkers B, 2009. Extrapolation in human health characterization: a probabilistic approach. Thesis. IRAS. Utrecht University.

Brandsma SH, van der Ven LTM, de Boer J and Leonards PEG, 2009. Identification of hydroxylated metabolites of hexabromocyclododecane in wildlife and 28-days exposed Wistar rats. Environmental Science and Technology, 43, 6058-6063.

BSEF (Bromine Science and Environmental Forum), 2010. About Hexabromocyclododecane (HBCD). 2010. Available from http://www.bsef.com/our-substances/hbcd/about-hbcd/ (accessed in July 2011).

Budakowski W and Tomy G, 2003. Congener-specific analysis of hexabromocyclododecane by high-performance liquid chromatography/electrospray tandem mass spectrometry. Rapid Communications in Mass Spectrometry, 17, 1399-1404.

Cantón RF, Peijnenburg AA, Hoogenboom RL, Piersma AH, van der Ven LT, van den Berg M and Heneweer M, 2008. Subacute effects of hexabromocyclododecane (HBCD) on hepatic gene expression profiles in rats. Toxicology and Applied Pharmacology, 231, 267-272.

Capen CC, 1997. Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. Toxicologic Pathology, 25, 39-48.

Cheaib Z, Grandjean D, Kupper T and de Alencastro LF, 2009. Brominated flame retardants in fish of Lake Geneva (Switzerland). Bulletin of Environmental Contamination and Toxicology, 82, 522-527.

Chengelis CP, 2001. A 90-day oral (gavage) toxicity study of HBCD in rats. WIL-186012, Wil Research Laboratories, Inc., Ashland, Ohio, USA, pp 1527. (as cited in ECB, 2008).

Colles A, Koppen G, Hanot V, Nelen V, Dewolf MC, Noël E, Malisch R, Kotz A, Kypke K, Biot P, Vinkx C and Schoeters G, 2008. Fourth WHO-coordinated survey of human milk for persistent organic pollutants (POPs): Belgian results. Chemosphere, 73, 907-914.

Covaci A, Voorspoels S and de Boer J, 2003. Determination of brominated flame retardants, with emphasis on polybrominated diphenyl ethers (PBDEs) in environmental and human samples—a review. Environment International, 29, 735-756.

Covaci A, Gerecke AC, Law RJ, Voorspoels S, Kohler M, Heeb NV, Leslie H, Allchin CR and de Boer J, 2006. Hexabromocyclododecanes (HBCDs) in the environment and humans: A review. Environmental Science and Technology, 40, 3679-3688.

Covaci A, Voorspoels S, Ramos L, Neels H and Blust R, 2007. Recent developments in the analysis of brominated flame retardants and brominated natural compounds. Journal of Chromatography A, 1153, 145-171.

Covaci A, Roosens L, Dirtu AC, Waegeneers N, van Overmeire I, Neels H and Goeyens L, 2009. Brominated flame retardants in Belgian home-produced eggs: Levels and contamination sources. Science of the Total Environment, 407, 4387-4396.

Crofton KM, Howard JL, Moser VC, Gill MW, Reiter LW, Tilson HA and MacPhail RC, 1991. Interlaboratory comparison of motor activity experiments: implications for neurotoxicological assessments. Neurotoxicology and Teratology, 13, 599-609.

Davis JW, Gonsior SJ, Marty G and Ariano JM, 2005. The transformation of hexabromocyclododecane in aerobic and anaerobic soils and aquatic sediments. Water Research, 39, 1075-1084.

de Boer J, Allchin C, Law R, Zegers B and Boon JP, 2001. Method for the analysis of polybrominated diphenylethers in sediment and biota. Trends in Analytical Chemistry, 20, 591-599.

de Boer J and Wells DE, 2006. Pitfalls in the analysis of brominated flame retardants in environmental, human and food samples – including results of three international interlaboratory studies. Trends in Analytical Chemistry, 25, 364-372.

Dingemans MML, heusinkveld HJ, de Groot A, Bergman Å, van den Berg M and Westerink RHS, 2009. Hexabromocyclododecane inhibits depolarization-induced increase in intracellular calcium levels and neurotransmitter release in PC12 cells. Toxicological Sciences, 107, 490-497.

Dirtu AC and Covaci A, 2010. Estimation of daily intake of organohalogenated contaminants from food consumption and indoor dust ingestion in Romania. Environmental Science & Technology, 44, 6297-6304.

Drottar KR and Krueger HO. Hexabromocyclododecane (HBCD), 2000. A flow-through bioconcentration test with the rainbow trout (*Oncorhynchus mykiss*). Final report. 2000; 439A-111, Wildlife International Ltd, Easton, Maryland, USA, 1-137.

Dubuisson C, Lioret S, Touvier M, Dufour A, Calamassi-Tran G, Volatier JL and Lafay L, 2010. Trends in food and nutritional intakes of French adults from 1999 to 2007: results from the INCA surveys. British Journal of Nutrition, 103, 1035-1048.

EBFRIP (European Brominated Flame Retardant Industry Panel), 2009. An evaluation of hexabromocyclododecane (HBCD) for Persistent Organic Pollutant (POP) properties and the potential for adverse effects in the environment. Report submitted to EBFRIP. Authors: Arnot J, McCarty L, Armitage J, Toose-Reid L, Wania F, Cousins I. 214 pp.

ECB (European Chemicals Bureau), 2008. Risk assessment. Hexabromocyclododecane. CAS-No.: 25637-99-4. EINECS-No.: 247-148-4. Final report May 2008.

ECHA (European Chemical Agency), 2009a. Data on manufacture, import, export, uses and releases of HBCDD as well as information on potential alternatives to its use. ECHA_2008_SR04_HBCDD_report_12_01_2009.doc. 108 pp

ECHA (European Chemical Agency), 2009b. Member State Committee Support Document for identification of hexabromocyclododecane and all major diastereoisomers identified as a substance of very high concern. Available from http://echa.europa.eu/doc/candidate_list/svhc_supdoc_hbccd_publication.pdf

EFSA (European Food Safety Authority), 2006. Advice of the Scientific Panel CONTAM related to relevant chemical compounds in the group of brominated flame retardants for monitoring in feed and food, 2006. The EFSA Journal, 328, 1-4. Available from http://www.efsa.europa.eu/en/efsajournal/doc/328.pdf

EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Committee related to uncertainties in dietary exposure assessment. The EFSA Journal, 438, 1-54. Available from http://www.efsa.europa.eu/en/efsajournal/doc/438.pdf

EFSA (European Food Safety Authority), 2009. Use of the benchmark dose approach in risk assessment. Guidance of the Scientific Committee. The EFSA Journal, 1150, 1-72. Available from http://www.efsa.europa.eu/en/efsajournal/doc/1150.pdf

EFSA (European Food Safety Authority), 2010. Standard sample description for food and feed. EFSA Journal 8(1):1457, 54 pp. Available from http://www.efsa.europa.eu/en/efsajournal/doc/1457.pdf

EFSA (European Food Safety Authority), 2011a. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. The EFSA Journal, 9(3):2097, 34 pp. Available from http://www.efsa.europa.eu/en/efsajournal/doc/2097.pdf

EFSA (European Food Safety Authority), 2011b. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal, 9(3):1970, 27 pp. Available from http://www.efsa.europa.eu/en/efsajournal/doc/1970.pdf

Eggesbø M, Thomsen C, Jørgensen JV, Becher G, Odland JØ and Longnecker MP, 2011. Association between brominated flame retardants in human milk and thyroid-stimulating hormone (TSH) in neonates. Environmental Research, in press, doi:10.1016/j.envres.2011.05.004

Eljarrat E, de la Cal A, Raldua D, Duran C and Barcelo D, 2004. Occurrence and bioavailability of polybrominated diphenyl ethers and hexabromocyclododecane in sediment and fish from the Cinca River, a tributary of the Ebro River (Spain). Environmental Science and Technology, 38, 2603-2608.

Eljarrat E, de la Cal A, Raldua D, Duran C and Barceló D, 2005. Brominated flame retardants in *Alburnus alburnus* from Cinca River Basin (Spain). Environmental Pollution, 133, 501-508.

Eljarrat E, Guerra P, Martínez E, Farré M, Alvarez JG, López-Teijón M and Barceló D, 2009. Hexabromocyclododecane in human breast milk: levels and Enantiomeric patterns. Environmental Science and Technology, 43, 1940-1946.

Eljarrat E, 2010. Occurrence and behaviour of brominated flame retardants. IV International Workshop on pollutants in the environment. November 10-12, 2010, Rio de Janeiro, Brazil.

EMEP (European Monitoring and Evaluation Programme), 2009. EMEP contribution to the preparatory work for the review of the CLRTAP protocol on persistend organic pollutants. New substances: Model assessment of potential for long-range transboundary atmospheric transport and persistence of Hexabromocyclododecane (HBCDD). Authors: Vulykh N, Rozovskaya O, Shatalov V. EMEP MSC-E Information Note 4/2009.

Environment Canada, 2010. Draft Screening Assessment Cyclododecane, 1,2,5,6,9,10-hexabromo-Chemical Abstracts Service Registry Number 3194-55-6. Environment Canada. Health Canada. August 2010.

Ema M, Fujii S, Hirata-Koizumi and Matsumoto M, 2008. Two-generation reproductive toxicity study of the flame retardant hexabromocyclododecane in rats. Reproductive Toxicology, 25, 335-351.

Engelhardt D and Hoffman HD, 2000. Cytogenetic study in vivo with of hexabromocyclododecane in the mouse micronucleus test after two intraperitoneal administrations. BASF, Ludwigshafen, Germany (as cited in ECB, 2008).

Eriksson P, Fischer C, Wallin M, Jakobsson E and Fredriksson A, 2006. Impaired behaviour, learning and memory, in adult mice neonatally exposed to hexabromocyclododecane (HBCDD). Environmental Toxicology and Pharmacology, 21, 317-322.

Fängström B, Athanassiadis I, Odsjö T, Norén K and Bergman Å, 2008. Temporal trends of polybrominated diphenyl ethers and hexabromocyclododecane in milk from Stockholm mothers, 1980-2004. Molecular Nutrition and Food Research, 52, 187-193.

Fång J, 2007. Separation and NMR-spectroscopy characterisation of technical grade hexabromocyclododecane isomers. Master thesis, Stockholm University, Environmental Chemistry.

Fernandes A, Dicks P, Mortimer D, Gem M, Smith F, Driffield M, White S and Rose M, 2008. Brominated and chlorinated dioxins, PCBs and brominated flame retardants in Scottish shellfish: methodology, occurrence and human dietary exposure. Molecular Nutrition & Food Research, 52, 238-249.

Fery Y, Buschauer I, Saliz C, Lang P and Schrenk D, 2009. Technical pentabromodiphenyl ether and hexabromocyclododecane as activators of the pregnane-X-receptor (PXR). Toxicology, 264, 458-51.

Fery Y, Mueller SO and Schrenk D, 2010. Development of stably transfected human and rat hepatoma cell lines for the species-specific assessment of xenobiotic response enhancer module (XREM)-dependent induction of drug metabolism. Toxicology, 277, 11-19.

FSA (Food Standards Agency), 2006. Brominated Chemicals: UK dietary intake. 10/06. June 2006.

Gerecke AC, Kohler M, Zennegg M, Schmid P and Heeb NV, 2003. Detection of α-isomer dominated HBCD (hexabromocyclododecane) in Swiss fish at levels comparable to PBDEs (polybrominated diphenyl ethers). Organohalogen Compounds, 61, 155-158.

Germer S, Piersma AH, van der Ven L, Kamyschnikow A, Fery Y, Schmitz H-J and Schrenk D, 2006. Subacute effects of the brominated flame retardants hexabromocyclododecane and tertabromobisphenol A on hepatic cytochrome P450 levels in rats. Toxicology, 218, 229-236.

Geyer HJ, Schramm KW, Darnerud PO, Aune M, Anton Feicht E, Fried KW, Henkelmann B, Lenoir D, Schmid P and McDonald TA, 2004. Terminal elimination half-lives of the brominated flame retardants TBBPA, HBCD, and lower brominated PBDEs in humans. Organohalogen Compounds, 66, 3867-3872.

Gómara B, Lebrón-Aguilar R, Quintanilla-López JE and González MJ, 2007. Development of a new method for the enantiomer specific determination of HBCD using an ion trap mass spectrometer. Analytica Chimica Acta, 605, 53-60.

Goscinny S, Vandevijvere S, Maleki M, van Overmeire I, Windal I, Hanot V, Blaude MN, Vleminckx C and van Loco J, 2011. Dietary intake of hexabromocyclododecane diastereoisomers (α-, β-, and γ-HBCD) in the Belgian adult population. Chemosphere, 84, 279-288.

Goss KU, Arp HPH, Bronner G and Niederer C, 2008. Partition behaviour of hexachlorocyclohexane isomers. Journal of Chemical and Engineering Data, 53, 750-754.

Graham MJ and Lake BG, 2008. Induction of drug metabolism: species differences and toxicological relevance. Toxicology, 254, 184-191.

Great Lakes Chemical Corporation, 2005. Material Safety Data Sheet. Great Lakes CD-75-P, CD-75PM and CD-75PC. West Lafayette, Indiana: Great Lakes Chemical Corporation. MSDS Number: 00177. Effective Date: 10/14/2005. 7 pp.

Gudi R and Schadly EH, 1996. Chromosome aberrations in human peripheral blood lymphocytes. Chemical Manufacturers Association. (not published) (as cited in ECB, 2008).

Hamers T, Kamstra JH, Sonneveld E, Murk AJ, Kester MHA, Andersson PL, legler J and Bruwer A, 2006. *In vitro* profiling of the endocrine-disrupting potency of brominated flame retardants. Toxicological Sciences, 92, 157-173.

Harju M, Heimstad ES, Herzke D, Sandaanger T, Posner S and Wania F, 2009. Current state of knowledge and monitoring requirements: Emerging "new" Brominated flame retardants in flame retarded products and the environment, Norwegian Pollution Control Authority, 1-114.

Harrad S, Ibarra C, Diamond M, Melymuk L and Robson M, 2008. Polybrominated diphenyl ethers in domestic indoor dust from Canada, New Zealand, United Kingdom and United States. Environment International, 34, 232-238.

Harrad S, Abdallah MAE and Covaci A, 2009a. Causes of variability in concentrations and diastereomer patterns of hexabromocyclododecanes in indoor dust. Environmental International, 35, 573-579.

Harrad S, Abdallah MAE, Rose NL, Turner SD, Simon D and Davidson TA, 2009b. Current-Use Brominated Flame Betardants in Water, Sediment, and Fish from English Lakes. Environmental Science & Technology, 43, 9077-9083.

Harrad S, Goosey E, Desborough J, Abdallah MA-E, Roosens L and Covaci A, 2010. Dust from U.K. Primary School Classrooms and Daycare Centers: The Significance of Dust As a Pathway of Exposure of Young U.K. Children to Brominated Flame Retardants and Polychlorinated Biphenyls. Environmental Science and Technology, 44, 4198-4202.

Harrad S and Abdallah MAE, 2011. Brominated flame retardants in dust from UK cars – within vehicle spatial variability, evidence for degradation and exposure implications. Chemosphere, 82, 1240-1245.

Haseman JK, Huff J and Boorman, GA, 1984. Use of historical control data in carcinogenicity studies in rodents. Toxicologic Pathology, 12, 126-135.

Haug LS, Thomsen C, Liane VH and Becher G, 2008. Comparison of GC and LC determinations of hexabromocyclododecane in biological samples - Results from two interlaboratory comparison studies. Chemosphere, 71, 1087-1092.

Haukås M, Hylland K, Nygård T, Berge JA and Mariussen E, 2010. Diastereomer-specific bioaccumulation of hexabromocyclododecane (HBCD) in a coastal food web, Western Norway, 2010. Science of the Total Environment, 408, 5910-5916.

Heeb NV, Schweizer WB, Mattrel P, Haag R, Gerecke AC, Kohler M, Schmid P, Zennegg M and Wolfensberger M, 2007. Solid-state conformations and absolute configurations of (+) and (-) α-, β-, and γ-hexabromocyclododecanes (HBCDs). Chemosphere, 68, 940-950.

Helleday T, Tuominen K-L, Bergman Å and Jenssen D, 1999. Brominated flame retardants induce intragenic recombination in mammalian cells. Mutation Research, 439, 137-147.

Hill RN, Crisp TM, Hurley PM, Rosenthal SL and Singh DV, 1998. Risk assessment of thyroid follicular cell tumors. Environmental Health Perspectives, 106, 447-457.

Hinkson and Whalen, 2009. Hexabromocyclododecane decreases the lytic function and ATP levels of human natural killer cells. Journal of Applied Toxicology, 29, 656-661.

Holson RR, Freshwater L, Maurissen JP, Moser VC and Phang W, 2008. Statistical issues and techniques appropriate for developmental neurotoxicity testing: a report from the ILSI Research Foundation/Risk Science Institute expert working group on neurodevelopmental endpoints. Neurotoxicology and Teratology, 30, 326-348.

Horpestad Liane V and Becher G, 2010. Interlaboratory comparison on POPs in food 2010. Eleventh round of an international study. Norwegian Institute of Public Health. Rapport 2010:5, 494 pp. Available from http://www.fhi.no/dokumenter/7714ee1951.pdf.

Hu X, Hu D and Xu Y, 2009. Effects of tetrabrominated diphenyl ether and hexabromocyclododecanes in single and complex exposure to hepatoma HepG2 cells. Environmental Toxicology and Pharmacology, 27, 327-337.

Huybrechts I, Sioen I, Boon PE, Ruprich J, Lafay L, Turrini A, Amiano P, Hirvonen T, De Neve M, Arcella D, Moschandreas J, Westerlund A, Ribas-Barba L, Hilbig A, Papoutsou S, Christensen T, Oltarzewski M, Virtanen S, Rehurkova I, Azpiri M, Sette S, Kersting M, Walkiewicz A, Serra-Majem L, Volatier JL, Trolle E, Tornaritis M, Busk L, Kafatos A, Fabiansson S, De Henauw S and van Klaveren J, in press. Dietary Exposure Assessments for Children in Europe (the EXPOCHI project): rationale, methods and design. Archives of Public Health, in press.

Ibhazehiebo K, Iwasaki T, Shimokawa N and Koibuchi N, 2011a. 1,2,5,6,9,10-αHexabromocyclododecane (HBCD) impairs thyroid hormone-induced dendrite arborization of Purkinje cells and suppresses thyroid hormone receptor-mediated transcription. Cerebellum, 10, 22-31.

Ibhazehiebo K, Iwasaki T, Xu M, Shimokawa N, Koibuchi N, 2011b. Brain-derived neurotrophic factor (BDNF) ameliorates the suppression of thyroid hormone-induced granule cell neurite extension by hexabromocyclododecane (HBCD). Neuroscience Letters 493, 1-7.

IOM (Insitute of Medicine), 1991. Institute of Medicine of the U.S. National Academies of Sciences. Nutrition During Lactation. National Academy Press. Washington, D.C.

Janak K, Covaci A, Voorspoels S and Becher G, 2005. Hexabromocyclododecane in marine species from the Western Scheldt Estuary: Diastereoisomer- and enantiomer-specific accumulation. Environmental Science and Technology, 39, 1987-1994.

Kajiwara N, Sueoka M, Ohiwa T and Takigami H, 2009. Determination of flame-retardant hexabromocyclododecane diastereomers in textiles. Chemosphere, 74, 1485-1489.

Kalantzi OI, Geens T, Covaci A and Siskos PA, 2011. Distribution of polybrominated diphenyl ether (PBDEs) and other persistent organic pollutants in human serum from Greece. Environment International, 37, 349-353.

Kersting M, Alexy U, Sichert-Hellert W, Manz F and Schoch G, 1998. Measured consumption of commercial infant food products in German infants: results from the DONALD study. Dortmund Nutritional and Anthropometrical Longitudinally Designed. Journal of Pediatric Gastroenterology and Nutrition, 27, 547-552.

Knutsen HK, Kvalem HE, Thomsen C, Froshaug M, Haugen M, Becher G, Alexander J and Meltzer HM, 2008. Dietary exposure to brominated flame retardants correlates with male blood levels in a selected group of Norwegians with a wide range of seafood consumption. Molecular Nutrition and Food Research, 52, 217-227.

Koppen R, Becker R, Esslinger S and Nehls I, 2010. Enantiomer-specific analysis of hexabromocyclododecane in fish from Etnefjorden (Norway). Chemosphere, 80, 1241-1245.

Koeppen R, Becker R, Emmerling F, Jung C and Nehls I, 2007. Enantioselective preparative HPLC separation of the HBCD-stereoisomers from the technical product and their absolute structure elucidation using X-Ray crystallography. Chirality, 19, 214-222.

Kurokawa Y, Inoue T, Uchida Y and Momma J., 2004. Carcinogenesis test of flame retarder hexabromocyclododecane in mice. M. Hardy, Albemarle Corporation, personal communication. 1984; Department of Toxicology, National Public Health Research Institute, Biological Safety Test and Research Centre. (not published) (as cited in ECB, 2008).

Law RJ, Kohler M, Heeb NV, Gerecke AC, Schmid P, Voorspoels S, Covaci A, Becher G, Janak K and Thomsen C, 2005. Hexabromocyclododecane challenges scientists and regulators. Environmental Science and Technology, 39, 281A-287A.

Law K, Palace VP, Halldorson T, Danell R, Wautier K, Evans B, Alaee M, Marvin C and Gregg TT, 2006. Dietary accumulation of hexabromocyclododecane diastereoisomers in juvenile rainbow trout (*Oncorhynchus mykiss*) I: Bioaccumulation parameters and evidence of bioisomerization. Environmental Toxicology & Chemistry. 25, 1757-1761.

Law RJ, Herzke D, Harrad S, Morris S, Bersuder P and Allchin CR, 2008. Levels and trends of HBCD and BDEs in the European and Asian environments, with some information for other BFRs. Chemosphere, 73, 223-241.

Li Y, Zhou Q, Wang Y and Xie X, 2011. Fate of tetrabromobisphenol A and hexabromocyclododecane brominated flame retardants in soil and uptake by plants. Chemosphere, 82, 204-209.

Lignell S, Glyn A, Darneru PO and Aune M, 2008. Temporal trends of PBDEs in mother's milk from Uppsala county, Sweden. Organohalogen Compounds, 70, 2123-2126.

Lignell S, Aune M, Isaksson M, Redeby J, Darnerud PO and Glynn A, 2011. BDE-209 in blood serum from first-time mothers in Uppsala – temporal trend 1996-2010. Report to the Swedish Environmental Protection Agency, 2011-03-31.

Lilienthal H, van der Ven L, Hack A, Roth-Härer A, Piersma A and Vos J, 2009. Neurobehavioral effects in relation to endocrine alterations caused by exposure to brominated flame retardants in rats – comparison to polychlorinated biphenyls. Human and Ecological Risk Assessment: An International Journal, 15, 76-86.

Lioret S, Dubuisson C, Dufour A, Touvier M, Calamassi-Tran G, Maire B, Volatier JL and Lafay L, 2010. Trends in food intake in French children from 1999 to 2007: results from the INCA (Etude Individuelle Nationale des Consommations Alimentaires) dietary surveys. British Jornal of Nutrition, 103, 585-601.

Lundstedt S, 2009. Emission, omvandling och bildning av bromerade ämnen vid olycksbränder (in Swedish). Report for the Swedish Rescue Service Agency (Räddningsverket). Umeå University, Umeå, Sweden.

Manolagas SC, 2000. Birth and death of bone cells: Basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocrine Reviews, 21, 115-137.

Manø S, Herzke D and Schlabach M, 2008. Nye miljøgifter i luft — 2007. Report 1031/2008. Oslo, Norway: Norwegian Pollution Control Authority (SFT). 60 pp. Available from http://www.klif.no/publikasjoner/2418/ta2418.pdf.

Mariussen E and Fonnum F, 2003. The effect of brominated flame retardants on neurotransmitter uptake into rat brain synaptosomes and vesicles. Neurochemistry International, 43, 533-542.

Mariussen E, Haukås M, Arp HPH, Goss K-U, Borgen A, Sandanger TM, 2010. Relevance of 1,2,5,6,9,10-hexabromocyclododecane diastereomer structure on partitioning properties, column-retention and clean-up procedures. Journal of Chromatography A, 1217, 1441-1446.

Marvin CH, Macinnis G, Alaee M, Arsenault G and Tomy GT, 2007. Factors influencing enantiomeric fractions of hexabromocyclododecane measured using liquid chromatography/tandem mass spectrometry. Rapid Communications in Mass Spectrometry, 21, 1925-1930.

MacGregor JA and Nixon WB, 2004. Determination of water solubility of hexabromocyclododecane (HBCD) using a generator column method. 2004; pp 52. Wildlife International, Ltd., Easton, Maryland, USA. (as cited by ECB, 2008).

McHugh B, Poole R, Corcoran J, Anninou P, Boyle B, Joyce E, Barry Foley M and McGovern E, 2010. The occurrence of persistent chlorinated and brominated organic contaminants in the European eel (*Anguilla anguilla*) in Irish waters. Chemosphere, 79, 305-313.

Meijer L, Weiss J, Vanvelzen M, Brouwer A, Bergman Å and Sauer PJJ, 2008. Serum concentrations of neutral and phenolic organohalogens in pregnant women and some of their infants in the Netherlands. Environmental Science and Technology, 42, 3428-3433.

Miller MD, Crofton KM, Rice DC and Zoeller RT, 2009. Thyroid-Disrupting Chemicals: Interpreting Upstream Biomarkers of Adverse Outcomes. Environmental Health Perspectives, 117, 1033-1041.

Moerbeek M, Piersma AH and Slob W, 2004. A comparison of three methods for calculating confidence intervals for the benchmark dose. Risk Analysis, 24, 31-40.

Morf LS, Buser AM, Taverna R, Bader H-P, Scheidegger R, 2008. Dynamic substance flow analysis as a valuable risk evaluation tool - a case study for brominated flame retardants as an example of potential endocrine disrupters. Chimia, 62, 424-431.

Morris S, Bersuder P, Allchin CR, Zegers B, Boon JP, Leonards PEG and de Boer J, 2006. Determination of the brominated flame retardant, hexabromocyclodocane, in sediments and biota by liquid chromatography-electrospray ionisation mass spectrometry. Trends in Analytical Chemistry, 25, 343-349.

Murai T, Kawasaki H and Kanoh S, 1985. Studies on the toxicity of insecticides and food additives in pregnant rats (7). Fetal toxicity of hexabromocyclododekane. Oyo Yakuri, 29, 981-986.

Nakagawa R, Murata S, Ashizuka Y, Shintani Y, Hori T and Tsutsumi T, 2010. Hexabromocyclododecane determination in seafood samples collected from Japanese coastal areas. Chemosphere, 81, 445-452.

OECD 2007. SIDS Initial Assessment Profile for CAS No. 25637-99-4, 3194-55-6, Hexabromocyclododecane (HBCDD). SIAM 24, 19-20 April 2007, 4 pp. Available from http://webnet.oecd.org/Hpv/UI/handler.axd?id=ea58ac11-e090-4b24-b281-200ae351686c.

Onyango AW, Receveur O and Esrey SA, 2002. The contribution of breast milk to toddler diets in western Kenya. Bulletin of the World Health Organization, 80, 292-299.

Ortiz X, Guerra P, Díaz-Ferrero J, Eljarrat E and Barceló D, 2011. Diastereoisomer- and enantiomer-specific determination of hexabromocyclododecane in fish oil for food and feed. Chemosphere, 82, 739-744.

Örn U and Bergman Å, 2004. An attempt to assess the present commercial production of brominated flame retardants. The Third International Workshop on Brominated Flame Retardants, Toronto, Canada, 467-472.

Päpke O, Petersen M, Ebsen P, Neugebauer F and Opel M, 2010. Brominated Flame Retardants in European Food Samples collected in 2007 to 2009. BFR 2010 Abstracts. Available from http://www.bfr2010.com/abstract-download/2010/bfr2010-abstracts-exposure-toxicology.html

Peled M, Scharia R and Sondack D, 1995. Thermal rearrangement of Hexabromo-cyclododecane (HBCD). In: Advances in Organobromine Chemistry II, Eds Desmurs JR, Gérard B and Goldstein MJ. Elsevier, Amsterdam, The Netherlands, p. 92-99.

Polder A, Thomsen C, Lindström G, Løken KB and Skaare JU, 2008. Levels and temporal trends of chlorinated pesticides, polychlorinated biphenyls and brominated flame retardants in individual human breast milk samples from Northern and Southern Norway. Chemosphere, 73, 14-23.

Pulkrabová J, Hajslova J, Poustka J and Kazda R, 2007. Fish as Biomonitors of Polybrominated Diphenyl Ethers and Hexabromocyclododecane in Czech Aquatic Ecosystems: Pollution of the Elbe River Basin. Environmental Health Perspectives, 115, 28-34.

Pulkrabová J, Hradkova P, Hajslova J, Poustka J, Napravnikova M and Polacek V, 2009. Brominated flame retardants and other organochlorine pollutants in human adipose tissue samples from the Czech Republic. Environment International, 35, 63-68.

QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe), 2009. Report BS-1 Brominated flame retardants Round 58 - Exercise 863 July 2009 to October 2009. -BS-1 Brominated flame retardants Round 56 - Exercise 835 January 2009 to April 2009. Available from www.quasimeme.org

QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe), 2010. Report on Round 60 - Exercise 881, January 2010 to April 2010, BS-1 Brominated flame retardants, 2010. Available from www.quasimeme.org

Rawn D, Sit D, Breakell K, Verigin V, Nicolidakis H, Feeley M and Ryan JJ, 2010. Hexabromocyclododecane (HBCDD) in Fish Oil Food Supplements. Organohalogen Compounds, 72, 393-396.

Rawn D, Sadler A, Lau B and Ryan JJ, 2009. Hexabromocyclododecane (HBCDD) in Egg Yolks from Market bound Canadian Chicken Eggs. Organohalogen Compounds, 71, 1240-1244.

Reistad T, Fonnum F and Mariussen E, 2006. Neurotoxicity of the pentabrominated diphenyl ether mixture, DE-71, and hexabromocyclododecane (HBCD) in rat cerebellar granule cells in vitro. Archives of Toxicology, 80, 785-796.

Remberger M, Sternbeck J, Palm A, Kaj L, Stromberg K and Brorstrom-Lunden E, 2004. The environmental occurrence of Hexabromocyclododecane in Sweden. Chemosphere, 54, 9-21.

Roosens L, Dirtu AC, Goemans G, Belpaire C, Gheorghe A, Neels H, Blust R and Covaci A, 2008. Brominated flame retardants and polychlorinated biphenyls in fish from the river Scheldt, Belgium. Environment International, 34, 976-983.

Roosens L, Abdallah MAE, Harrad S, Neels H and Covaci A, 2009. Exposure to Hexabromocyclododecanes (HBCDs) via dust ingestion, but not diet, correlates with concentrations in human serum: preliminary results. Environmental Health Perspectives, 117, 1707-1712.

Roosens L, D'Hollander W, Bervoets L, Reynders H, Van Campenhout K, Cornelis C, Van Den Heuvel R, Koppen G and Covaci A, 2010a. Brominated flame retardants and perfluorinated chemicals, two groups of persistent contaminants in Belgian human blood and milk. Environmental Pollution, 158, 2546-2552.

Roosens L, Cornelis C, D'Hollander W, Bervoets L, Reynders H, van Campenhout K, van den Heuvel R, Neels H and Covaci A, 2010b. Exposure of the Flemish population to brominated flame retardants: Model and risk assessment. Environment International, 36, 368-376.

Roosens L, Geeraerts C, Belpaire C, Van Pelt I, Neels H and Covaci A, 2010c. Spatial variations in the levels and isomeric patterns of PBDEs and HBCDs in the European eel in Flanders. Environment International, 36, 415-423

Rylander L, Stromberg U and Hagmar L, 1995. Decreased birthweight among infants born to women with a high dietary intake of fish contaminated with persistent organochlorine compounds. Scandinavian Journal of Work and Environmental Health, 21, 368-375.

Saegusa Y, Fujimoto H, Woo G-H, Inoue K, Takahashi M, Mitsumori K, Hirose M, Nishikawa A and Shibutani M, 2009. Developmental toxicity of brominated flame retardants, tetrabromobisphenol A and 1,2,5,6,9,10-hexabromocyclododecane, in rat offspring after maternal exposure from mid-gestation through lactation. Reproductive Toxicology, 28, 456-467.

Säll L, 2010. Exploration of management options for Hexabromocyclododecane (HBCD). Paper for the 8th meeting of the UNECE CLRTAP Task Force on Persistent Organic Pollutants, Montreal 18-20 May 2010. Climate and Pollution Agency in Norway, 18 August 2010 (updated version), 48 pp.

Schechter A, Haffner D, Colacino J, Patel K, Päpke O, Opel M and Birnbaum L, 2010. Polybrominated diphenyl ethers (PBDEs) and hexabromocyclodecane (HBCD) in composite U.S. food samples. Environmental Health Perspectives, 118, 357-362.

Schlabach M, Mariussen E, Borgen A, Dye C, Enge E-K, Steinnes E, Green N and Mohn H, 2002. Kartlegging av bromerte flammehemmere og klorerte parafiner. Norsk institutt for luftforskning (NILU), Kjeller, Norway, Rapport 62/2002, 71 pp. Available from http://www.klif.no/publikasjoner/overvaking/1924/ta1924.pdf

Schuetz EG, Brimer C and Shuetz JD, 1998. Environmental xenobiotics and the antihormones cyproterone acetate and spironolactone use the nuclear hormone pregnenolone X receptor to activate the CYP3A23 hormone response element. Molecular Pharmacology, 54, 1113-1117.

Sellström U, Kierkegaards A, de Wit C and Jansson B, 1998. Polybrominated diphenyl ethers and hexabromocyclododecane in sediment and fish from a Swedish river. Environmental Technology and Chemistry, 17, 1065-1072.

Shaw SD, Berger ML, Brenner D, Lohmann N and Päpke O, 2008. Bioaccumulation of PBDEs and HBCD in teh northest Atlantic marine food web. Organohalogen Compounds, 70, 841-845.

Simmon VF, Poole DC, Newell GW and Skinner WA, 1976. In vitro microbiological mutagenicity studies of four CIBA-GEIGY corporation compounds. Prepared for CIBAGeigy Corporation (not published).; 5702, SRI Project LSC. (as cited in ECB, 2008).

Slob W, 2002. Dose-response modeling of continuous endpoints. Toxicological Sciences, 66, 298-312.

Slob W and Pieters MN, 1998. A probabilistic approach for deriving acceptable human intake limits and human health risks from toxicological studies: general framework. Risk Analysis, 18, 787-798.

Söderström G and Marklund S, 1999. Fire of a flame retarded TV. Organohalogen Compounds, 41, 469-472.

Stump DG, 1999. Prenatal developmental toxicity study of hexabromocyclododecane (HBCD) in rats.; pp 410. WIL Research Laboratories, Inc., Ashland, Ohio, USA. (as cited in ECB, 2008).

Svensson BG, Nilsson A, Jonsson E, Schutz A, Akesson B and Hagmar L, 1995. Fish consumption and exposure to persistent organochlorine compounds, mercury, selenium and methylamines among Swedish fishermen. Scandinavian Journal of Work and Environmental Health, 21, 96-105.

Szabo DT, Diliberto JJ, Hakk H, Huwe JK and Birnbaum LS, 2011. Toxicokinetics of the Flame Retardant Hexabromocyclododecane Alpha: Effect of Dose, Timing, Route, Repeated Exposure, and Metabolism. Toxicological Sciences, 121, 234-244.

Szabo DT, Diliberto JJ, Hakk H, Huwe JK and Birnbaum LS, 2010. Toxicokinetics of the flame retardant hexabromocyclododecane gamma: effect of dose, timing, route, repeated exposure, and metabolism. Toxicological Sciences, 117, 282-293.

Tabin, CJ, 1991. Retinoids, homoboxes, and growth factors: toward molecular models for limb development. Cell, 66, 199-217.

Thomsen C, Stigum H, Frøshaug M, Broadwell SL, Becher G, Eggesbø M, 2010. Determinants of brominated flame retardants in breast milk from a large scale Norwegian study. Environment International, 36, 68-74.

Thomsen C, Molander P, Daae HL, Janák K, Frøshaug M, Liane VH, Thorud S, Becher G and Dybing E, 2007. Occupational Exposure to Hexabromocyclododecane at an Industrial Plant. Environmental Science and Technology, 41, 5210-5216.

Thomsen C, Knutsen HK, Liane VH, Frøshaug M, Kvalem HE, Haugen M, Meltzer HM, Alexander J and Becher G, 2008. Consumption of fish from a contaminated lake strongly affects the concentrations of polybrominated diphenyl ethers and hexabromocyclododecane in serum. Molecular Nutrition and Food Research, 52, 228-237.

Tomy GT, Halldorson T, Danell R, Law K, Arsenault G, Alaee M, MacInnis G and Marvin CH, 2005. Refinements to the diastereoisomer-specific method for the analysis of hexabromocyclododecane. Rapid Communications in Mass Spectrometry, 19, 2819-2826.

Törnkvist A, Glynn A, Aune M, Darnerud PO, Ankarberg HE, 2011. PCDD/F, PCB, PBDE, HBCD and chlorinated pesticides in a Swedish market basket from 2005 – Levels and dietary intake estimations. Chemosphere, 83, 193-199.

UNEP (United Nations Environment Programme), 2010. Report of the Persistent Organic Pollutants Review Committee on the work of its sixth meeting. Addendum 2. UNEP/POPS/POPRC.6/13/Add.2

US-EPA (US Environmental Protection Agency), 1990. Ames test with hexabromides. Letter from BASF. 86-900000379, EPA/OTS Doc. (as cited in ECB, 2008).

US-EPA (US Environmental Protection Agency), 2008. Initial Risk-Based Prioritization of High Production Volume Chemicals. Chemical/Category: Hexabromocyclododecane (HBCD). Risk-Based Prioritization Document 3/18/2008.

van der Molen GW, 1998. A Physiologicallly-based mathematical model for long-term kinetics of dioxins and furans in humans, Thesis Utrecht University, Utrecht, The Netherlands.

van der Ven L, Verhoef A, van de Kuil T, Slob W, Leonards PEG, Visser TJ, Hamers T, Herlin M, Håkansson H, Olausson H, Piersma AH and Vos JG, 2006. A 28-day oral dose toxicity study enhanced to detect endocrine effects of hexabromocyclododecane in Wistar rats. Toxicological Sciences, 94, 281-292.

van der Ven L, van de Kuil T, Leonards PEG, Slob W, Lilienthal H, Litens H, Herlin M, Håkansson H, Cantón RF, van den Berg M, Visser TJ, van Loveren H, Vos JG and Piersma AH, 2009. Endocrine effects of hexabromocyclododecane (HBCD) in a one-generation reproduction study in Wistar rats. Toxicology Letters, 185, 51-62.

van Leeuwen SPJ, 2009. Fluorinated, Chlorinated and Brominated Contaminants in Fish for Human Consumption- Methods and Measurements. Thesis, Vrije Universiteit, Amsterdam, The Netherlands.

van Leeuwen SPJ and de Boer J, 2008. Brominated flame retardants in fish and shellfish - levels and contribution of fish consumption to dietary exposure of Dutch citizens to HBCD. Molecular Nutrition and Food Research, 52, 194-203.

van Leeuwen, SPJ, van Velzen M, Swart K, van der Veen I, Traag W, Spanjer M, Scholten J, van Rhijn H and de Boer J, 2008. Contaminants in popular farmed fish consumed in The Netherlands and their levels in fish feed, Report R-08/03, Institute for Environmental Studies – VU University Amsterdam, The Netherlands.

Veith GD, DeFoe DL and Bergstedt BV, 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. Journal of the Fisheries Research Board of Canada, 36, 1040-1048.

Vorkamp K, Rigét FF, Bossi R and Dietz R, 2011. Temporal trends of hexabromocyclododecane, polybrominated diphenyl ethers and polychlorinated biphenyls in ringed seals from East Greenland. Environmental Science & Technology, 45, 1243-1249.

Vos JG, Becher G, van den Berg M, de Boer J and Leonards PEG, 2003. Brominated flame retardants and endocrine disruption. Pure and Applied Chemistry, 75, 2039-2046.

Watanabe W, Shimizu T, Sawamura R, Hino A, Konno K, Hirose A and Kurokawa A, 2010. Effects of tetrabromobisphenol A, a brominated flame retardant, on the immune response to respiratory syncytial virus infection in mice. International Immunopharmacology, 10, 393-397.

Weiss J, Wallin E, Axmon A, Jönsson BA, Akesson H, Janák K, Hagmar L and Bergman Å, 2006. Hydroxy-PCBs, PBDEs, and HBCDDs in serum from an elderly population of Swedish fishermen's wives and associations with bone density. Environmental Science and Technology, 40, 6282-6289.

Winter-Sorkina R de, Bakker MI, van Dinkersgoed G and van Klaveren JD, 2003. Dietary intake of brominated flame retardants by the Dutch population. RIVM report 310305001/2003.

WHO (World Health Organisation), 1997. Environmental Health Criteria 192. Flame Retardants: A General Introduction. Geneva, Switzerland: International Program on Chemical Safety, World Health Organization.

WHO (World Health Organisation), 2003. Instructions for electronic submission of data on chemical contaminants in food and the diet. Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food). 160 pp. Available from http://www.who.int/foodsafety/publications/chem/en/gemsmanual.pdf.

WHO/IPCS (World Health Organisation/International Program on Chemical Safety), 2008. Uncertainty and data quality in exposure assessment. Harmonisation project document No. 6. 175 pp. Available from http://www.who.int/ipcs/publications/methods/harmonization/exposure_assessment.pdf.

Yamada-Pkabe T, Sakai H, Kashima Y and Yamada-Okabe H, 2005. Modulation at a cellular level of the thyroid hormone receptor-*mediated gene expression by 1,2,5,6,9,10-hexabromocyclododecane (HBCD), 4,4'-diiodobiphenyl (DIB), and nitrofen (NIP). Toxicology Letters, 155, 127-133.

Zhang X, Yang F, Xu C, Liu W, Wen S and Xu Y, 2008. Cytotoxicity evaluation of three pairs of hexabromocyclododecane (HBCD) enantiomers on Hep G2 cell. Toxicology in Vitro, 22, 1520-1527.

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K and Speck W, 1987. Salmonella mutagenicity tests: III. Results from the testing of 255 chemicals. Environmental Mutagenesis, Supplement, 9, 1-110.

Zeller H and Kirsch P, 1969. Hexabromocyclododecane: 28-day feeding trials with rats. BASF (not published) (as cited in ECB, 2008).

APPENDICES

A. STRUCTURE AND CAS NUMBERS OF HBCDDS

(+/-)-α-HBCD, 1,2,5,6,9,10-hexabromo-(1R,2R,5S,6R,9R,10S)-rel-cyclododecane (CAS: 134237-50-6)

(+/-)-β-HBCD 1,2,5,6,9,10-hexabromo-(1R,2S,5R,6R,9R,10S)-rel-cyclododecane (CAS: 134237-51-7)

(+/-)-γ-HBCD 1,2,5,6,9,10-hexabromo-(1R,2R,5R,6S,9S,10R)-rel-cyclododecane (CAS: 134237-52-8)

1,2,5,6,9,10-Hexabromo-(1R,2S,5R,6R,9R,10R)-cyclododecane (CAS: 878049-08-2);

1,2,5,6,9,10-hexabromo-, (1S,2S,5R,6R,9R,10R)- cyclododecane (CAS: 878049-05-9);

1,2,5,6,9,10-hexabromo-(1R,2R,5R,6R,9R,10R)-cyclododecane (CAS: 878049-04-8);

1,2,5,6,9,10-hexabromo-(1R,2R,5R,6S,9S,10R)-cyclododecane (+) (CAS: 678970-17-7);

1,2,5,6,9,10-hexabromo-(1R,2S,5R,6S,9S,10S)-cyclododecane (+) (CAS: 678970-16-6);

1,2,5,6,9,10-hexabromo-(1R,2R,5S,6R,9R,10S)-cyclododecane (-) (CAS: 678970-15-5);

1,2,5,6,9,10-hexabromo-(1R,2S,5S,6S,9S,10R)-cyclododecane (-) (CAS: 169102-57-2);

1,2,5,6,9,10-hexabromo-(1R,2S,5S,6R,9S,10S)-cyclododecane (+), (CAS: 138257-19-9);

1,2,5,6,9,10-hexabromo-(1R,2R,5R,6S,9R,10S)-cyclododecane (-) (CAS: 138257-18-8);

1,2,5,6,9,10-hexabromo-, (1R,2R,5R,6S,9S,10S)-cyclododecane (CAS: 138257-17-7).

B. OCCURRENCE IN FISH

Table B1: Selection of data on HBCDD levels in fish and crustaceans in Europe (ng/g w.w.). Data concerns averages, unless ranges are given. p indicates that each sample was a pool of several individual fishes. n.r.: not reported.

Country	Location	Year	n	Sample	Tissue	Fat (%)	Total HBCDD	α-HBCDD	β-HBCDD	γ-HBCDD	Sum-HBCDD	Reference
ES	Cinca river	2002	23	Barbel (*Barbus barbus*)	Muscle	n.r.	ND-750	n.r.	n.r.	n.r.	n.r.	Eljarrat et al., 2004
ES	Cinca river	2002	23	Barbel	Liver	n.r.	ND-625	n.r.	n.r.	n.r.	n.r.	Eljarrat et al., 2004
CZ	Elbe river	2001-2003	5p	Barbel	Muscle	2.4-4.8	1.8-15.6	n.r.	n.r.	n.r.	n.r.	Pulkrabová et al., 2007
ES	Cinca river	2002	22	Bleak *(Alburnus alburnus)*	Whole fish	20-25	ND-1643	n.r.	n.r.	n.r.	n.r.	Eljarrat et al., 2005
CZ	Elbe river	2001-2003	6p	Bream (*Abramis brama)*	Muscle	2.1-4.6	0.8-7.4	n.r.	n.r.	n.r.	n.r.	Pulkrabová et al., 2007
UK	Skerne and Tees rivers	2002	n.r	Brown trout (*Salmo trutta trutta)*	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	<1.2-6758	Allchin and Morris, 2003
NO	Various	2002-2006	2	Caviar	n.r.	36.4	n.r.	<LOD	<LOD	<LOD	n.r.	Knutsen et al., 2008
CZ	Elbe river	2001-2003	6p	Chub *(Squalius cephalus)*	Muscle	1.9-3.2	1.3-4.1	nr	nr	nr	n.r.	Pulkrabová et al., 2007
NL		2003	2	Coalfish (*Pollachius pollachius*)	Muscle	1-1.1	<0.1-0.2	<0.1-0.2	<0.1	<0.2	n.r.	van Leeuwen and de Boer, 2008
NL		2003	2	Cod (*Gadus morhua)*	Muscle	0.7-0.9	<0.1	<0.1-<0.2	<0.1-<0.2	<0.2	n.r.	van Leeuwen and de Boer, 2008
NO	Various	2002-2006	1	Cod	Liver	55.7	n.r.	3.6	n.r.	n.r.	n.r.	Knutsen et al., 2008
NO	Various	2002-2006	3	Cod	Roe	6.8	n.r.	<LOD	<LOD	<LOD	n.r.	Knutsen et al., 2008
NO		2007-2008	1	Cod (F)	Muscle	n.r.	n.r.	<0.01	<0.02	<0.02	n.r.	van Leeuwen et al., 2008
NO	Various	2002-2006	6	Cod liver pate	Liver	31.2	n.r.	0.48	0.06	<LOD	n.r.	Knutsen et al., 2008
UK	Skerne and Tees rivers	<2003	n.r.	Eel (*Anguilla anguilla*)	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	40-10275	Allchin and Morris, 2003
NL		2003	14	Eel	Muscle	4.1-23	<0.1-210	<0.7-41	<0.7-1.0	<1.5-8.4	n.r.	van Leeuwen and de Boer, 2008
IRL	Various rivers	2005, 2007	3p	Eel	Muscle	14.3-20.9	1.2-2.2	n.r.	n.r.	n.r.	n.r.	McHugh et al., 2010
IRL	Various rivers	2005, 2007	2p	Eel	Muscle	9.2-16	15	6.0-8.9	0.2-0.5	0.2-0.5	n.r.	McHugh et al., 2010
IT, NL		2003	2	Eel (F)	Muscle	22-36	<0.4-<0.5	<0.7-<0.9	<0.7-<0.9	<1.4-<1.8	n.r.	van Leeuwen and de Boer, 2008

Table B1: Continued.

Country	Location	Year	n	Sample	Tissue	Fat (%)	Total HBCDD	α-HBCDD	β-HBCDD	γ-HBCDD	Sum-HBCDD	Reference
NL		2003	2	Flounder (*Platichthys flesus)*	Muscle	1.1-1.7	0.9-1.3	0.3-0.5	<0.1	<0.2	n.r.	van Leeuwen and de Boer, 2008
NL		2003	2	Haddock (*Melanogrammus aeglefinus*	Muscle	0.7-0.8	<0.1	<0.1-<0.2	<0.1-<0.2	<0.2	n.r.	van Leeuwen and de Boer, 2008
NL		2003	4	Herring (*Clupea harengus*)	Muscle	14-20	<0.1-2.7	1.0-2.0	<0.8-<0.9	<1-6-1.9	n.r.	van Leeuwen and de Boer, 2008
NO	Various	2002-2006	4	Herring	n.r.	11.4	n.r.	0.33	n.r.	n.r.	n.r.	Knutsen et al., 2008
CH	Lake Geneva	2004	9	Lake trout	Whole fish	6.4	10.8	n.r.	n.r.	n.r.	n.r.	Cheaib et al., 2009
NL		2003	3	Mackerel (*Scomber scombrus)*	Muscle	3.3-17	<0.5-2.1	<0.5-1.7	<0.5-1.0	<1.1-<1.9	n.r.	van Leeuwen and de Boer, 2008
NO	Various	2002-2006	6	Mackerel	n.r.	25.7	n.r.	0.49	0.03	0.04	n.r.	Knutsen et al., 2008
NO	Various	2002-2006	3	Mackerel in tomato sauce	n.r.	19.5	n.r.	0.53	<LOD	0.01	n.r.	Knutsen et al., 2008
NL		2003	2	Mussel	Flesh	2.1-2.2	0.2-0.9	<0.1	<0.1	<0.2	n.r.	van Leeuwen and de Boer, 2008
UK		2006	10	Mussel	Flesh	n.r.	n.r.	0.1-8.9	0.01-1.6	0.03-1.5	n.r.	Fernandes et al., 2008
UK		2006	5	Oyster	Flesh	n.r.	n.r.	0.3-1.0	0.04-0.2	0.03-0.15	n.r.	Fernandes et al., 2008
VN		2007-2008	7	Pangasius (F)	Muscle	1.9 +/- 0.9	n.r.	<0.01-0.03	<0.02-<0.07	<0.01-<0.07	n.r.	van Leeuwen et al., 2008
CZ	Elbe river	2001-2003	6p	Perch *(Perca fluviatilis)*	Muscle	0.6-1.0	0.4-1.6	n.r.	n.r.	n.r.	n.r.	Pulkrabová et al., 2007
NL		2003	2	Pike-perch (*Sander lucioperca)*	Muscle	0.9-1	<0.1	<0.1	<0.1	<0.2	n.r.	van Leeuwen and de Boer, 2008
NL		2003	2	Plaice (*Pleuronectes platessa)*	Muscle	0.9-1.3	<0.1	<0.1-<0.2	<0.1-<0.2	<0.2	n.r.	van Leeuwen and de Boer, 2008
Various		2007-2008	7	Salmon (F) (*Salmo salar*)	Muscle	14.5 +/- 3.7	n.r.	<0.1-0.3	<0.1-<0.3	<0.1-<0.3	n.r.	van Leeuwen et al., 2008
NO, UK		2003	2	Salmon (F)	Muscle	12	<0.1-1.3	<0.4-<0.5	<0.4-<0.5	<0.9-<1.0		van Leeuwen and de Boer, 2008
NO	Various	2002-2006	6	Salmon (F)	n.r.	14.8	n.r.	0.13	n.r.	n.r.	n.r.	Knutsen et al., 2008
NO	Various	2002-2006	5	Salmon, smoked	n.r.	11.1	n.r.	0.24	<LOD	0.01	n.r.	Knutsen et al., 2008
NO	Various	2002-2006	3	Sardines (*Sardina pilchardus)*	n.r.	29.1	n.r.	0.62	<LOD	0.01	n.r.	Knutsen et al, 2008
UK		2006	10	Scallops gonad	Flesh	n.r.	n.r.	0.06-1.8	0.01-0.24	0.01-0.16	n.r.	Fernandes et al., 2008

				(Pecten spp.*)*								

Table B1: Continued.

Country	Location	Year	n	Sample	Tissue	Fat (%)	Total HBCDD	α-HBCDD	β-HBCDD	γ-HBCDD	Sum-HBCDD	Reference
NL		2003	2	Shrimp	Flesh	2.1-2.3	<0.1	<0.5	<0.5	<1.0	n.r.	van Leeuwen and de Boer, 2008
NO	Various	2002-2006	2	Shrimp	n.r.	1.2	n.r.	0.01	<LOD	<LOD	n.r.	Knutsen et al., 2008
Various		2007-2008	6	Shrimp (F)	Flesh	1.2 +/- 0.5	n.r.	<0.02-0.7	<0.01-0.5	<0.01-<0.02	n.r.	van Leeuwen et al., 2008
NL		2003	2	Sole (*Sola sola*)	Muscle	1.1-1.2	<0.1	<0.1	<0.1	<0.2	n.r.	van Leeuwen and de Boer, 2008
Various		2007-2008	6	Tilapia (F) *(Oreochromis* spp.*)*	Muscle	3.1 +/- 1.2	n.r.	<0.01-<0.03	<0.02-<0.07	<0.02-<0.06	n.r.	van Leeuwen et al., 2008
Various		2007-2008	5	Trout (F)	Muscle	6.6 +/- 1.4	n.r.	0.05-0.1	<0.06-<0.09	<0.07-<0.09	n.r.	van Leeuwen et al., 2008
NO	Various	2002-2006	3	Trout (F)	n.r.	19.5	n.r.	0.65	0.02	0.05	n.r.	Knutsen et al., 2008

n: number of samples; p: each sample was a pool of several individual fishes; n.r.: not reported; LOD: limit of detection; CH: Switzerland; CZ: Czech Republic; ES: Spain; IRE: Ireland; IT: Italy; NL: The Netherlands; NO: Norway; UK: United Kingdom; VN: Vietnam.

Table B2: Selection of data on HBCDD levels in fish and crustaceans in Europe (in ng/g fat). Data concerns averages, unless ranges are given. p indicates that each sample was a pool of several individual fishes.

Country	Location	Year	n	Sample	Tissue	Fat (%)	Total HBCDD	α-HBCDD	β-HBCDD	γ-HBCDD	Sum-HBCDD	Reference
SE		2002	6	Herring (*Clupea harengus*)	Muscle	n.r.	4.0-18.3	n.r.	n.r.	n.r.	n.r.	Asplund et al., 2004
CH		<2003	6p	Whitefish (*Coregonus lavaretus*)	Muscle	1.5-7.2	25-210	54-210	<3-<15	<6-<33	n.r.	Gerecke et al., 2003
BE		2000-2006	50p	Eel (*Anguilla anguilla*)	Muscle	2.0-21.4	n.r.	n.r.	n.r.	n.r.	14-3530	Roosens et al., 2010c
BE	Scheldt river	2000-2006	10p	Eel	Muscle	0.9-20.1	n.r.	n.r.	n.r.	n.r.	2600-10100	Roosens et al., 2008
SE	Marine	1999-2000	6p	Herring (*Clupea harengus*)	Muscle	2.1-5.8	21-180	n.r.	n.r.	n.r.	n.r.	Remberger et al., 2004
NO	Etnefjorden	2006	20	Mackerel (*Scomber scombrus)*	Muscle	n.r.	n.r.	184.1	11.2	23.5	n.r.	Koppen et al., 2010
NO	Etnefjorden	2006	1	Cod *(Gadus morhua)*	Muscle	n.r.	n.r.	21250	8850	216.8	n.r.	Koppen et al., 2010
NO	Etnefjorden	2006	1	Thorny skate (*Amblyraja radiata)*	Muscle	n.r.	n.r.	1476.5	86.6	153.4	n.r.	Koppen et al., 2010
NO	Etnefjorden	2006	1	Pollack (*Pollachius pollachius*)	Muscle	n.r.	n.r.	1090.6	150.48	54.85	n.r.	Koppen et al., 2010
NO	Etnefjorden	2006	2	Flounder (*Platichthys flesus)*	Muscle	n.r.	n.r.	410.6	52.7	218.1	n.r.	Koppen et al., 2010
BE	Scheldt Estuarry	2001	2p	Shrimp	Whole	0.7	n.r.	28-38	n.r.	<2-18	n.r.	Janak et al., 2005
BE	Scheldt Estuarry	2001	2p	Eel (*Anguilla anguilla*)	Muscle	26	n.r.	7-27	n.r.	2-3	n.r.	Janak et al., 2005
BE	Scheldt Estuarry	2001	4p	Sole *(Sola sola)*	Muscle	1	n.r.	110-1100	n.r.	6-17	n.r.	Janak et al., 2005
BE	Scheldt Estuarry	2001	1p	Plaice *Pleuronectes platessa)*	Muscle	0.8	n.r.	38	n.r.	<2	n.r.	Janak et al., 2005
BE	Scheldt Estuarry	2001	3p	Bib (*Trisopterus luscus)*	Muscle	0.5	n.r.	53-97	n.r.	<3-43	n.r.	Janak et al., 2005
BE	Scheldt Estuarry	2001	3p	Whiting (*Merlangius merlangus)*	Muscle	0.4	n.r.	45-75	n.r.	<3-51	n.r.	Janak et al., 2005

Table B2: Continued.

Country	Location	Year	n	Sample	Tissue	Fat (%)	Total HBCDD	α-HBCDD	β-HBCDD	γ-HBCDD	Sum-HBCDD	Reference
UK	Various lakes	2008	1	Trout (rainbow) (*Oncorhynchus mykiss)*	Muscle	n.r.	n.r.	110	22	43	180	Harrad et al., 2009b
SE	River Viskan	1995	12	Pike (*Esox lucius)*	n.r.	0.46-0.79	<80-8000	n.r.	n.r.	n.r.	n.r.	Sellström et al., 1998
SE	Lake Skaresjon	1995	3	Pike	n.r.	0.65-1.09	<50-<90	n.r.	n.r.	n.r.	n.r.	Sellström et al., 1998

n: number of samples; p: each sample was a pool of several individual fishes; n.r.: not reported; BE: Belgium; CH: Switzerland; NO: Norway; SE: Sweden; UK: United Kingdom.

C. CONSUMPTION DATA

Table C1: Dietary surveys considered for the chronic dietary exposure assessment and number of subjects in the different age classes.

Country	Dietary survey[a]	Abbreviation[b]	Number of subjects							
			Total	Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
Belgium	Diet National 2004	BE/1	3118				584	1304	518	712
	Regional_Flanders	BE/2	661		36[c]	625				
Bulgaria	NUTRICHILD	BG	1721	860	428	433				
Cyprus	Childhealth	CY	303				303			
Czech Republic	SISP04	CZ	2353			389	298	1666		
Denmark	Danish_Dietary_Survey	DK	4120			490	479	2822	309	20[c]
Finland	DIPP	FI/1	1430		497	933				
	FINDIET_2007	FI/2	2038					1575	463	
	STRIP	FI/3	250			250				
France	INCA2	FR	4079			482	973	2276	264	84
Germany	DONALD_2006	DE/1	303		92	211				
	DONALD_2007	DE/2	311		85	226				
	DONALD_2008	DE/3	307		84	223				
	National_Nutrition_Survey_II	DE/4	13926				1011	10419	2006	490
Greece	Regional_Crete	GR	839			839				
Hungary	National_Repr_Surv	HU	1360					1074	206	80
Ireland	NSIFCS	IE	958					958		
Italy	INRAN_SCAI_2005_06	IT	3323	16[c]	36[c]	193	247	2313	290	228
Latvia	EFSA_TEST	LT	1965			189	470	1306		
Netherlands	DNFCS_2003	NL/1	750					750		
	VCP_kids	NL/2	1279		322	957				
Spain	AESAN	ES/1	410					410		
	AESAN_FIAB	ES/2	1067				86	981		
	NUT_INK05	ES/3	1050			399	651			
	enKid	ES/4	382		17[c]	156	209			
Sweden	Riksmaten_1997_98	SE/1	1210					1210		
	NFA	SE/2	2491			1473	1018			
United Kingdom	NDNS	UK	1724					1724		

BE: Belgium; BG: Bulgaria; CY: Cyprus; CZ: Czech Republic; DK: Denmark; FI: Finland; FR: France; DE: Germany; GR: Greece; HU; Hungary; IE: Ireland; IT: Italy; LV: Latvia; NL; The Netherlands; ES: Spain; SE: Sweden; UK: United Kingdom; (a): More information on the dietary surveys is given in the Guidance of EFSA 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b); (b): Abbreviations used consistently in all tables on exposure assessment; (c): The 95th percentile calculated over a number of observations lower than 60 require cautious interpretation as the results may not be statistically robust (EFSA, 2011b) and therefore for these dietary surveys/age classes the 95th percentile estimates will not be presented in the exposure assessment.

D. OCCURRENCE DATA

Table D1: Selection of occurrence means (ng/g w.w.) for the sum of the individual stereoisomers (α+β+γ) and the total HBCDD across ad-hoc food groups based on the FoodEx food classification system that are used for the chronic dietary exposure assessment. In view a more comprehensive linkage of consumption habits with HBCDDs occurrence data across food groups, assumptions have been done as reported in details in the footnotes.

Ad-hoc food groups for HBCDDs exposure assessment	FoodEx food groups	HBCDD-sum (α+β+γ)		Total HBCDD
		Type	Mean	Mean
Eggs and egg products	Eggs and egg products	LB	0.02	
	Egg-based meal (composite food e.g., omelette) [(a)]	UB	0.06	
Milk and dairy products	Milk and dairy products	LB	<0.01	
		UB	0.04	
Meat and meat products	Meat and meat products (including edible offal)	LB	0.01	
	Meat-based meals (composite food)[(b)]	UB	0.04	
Fish meat and products	Fish meat	LB		0.48
		UB		0.68
	Fish and other seafood (including amphibians, reptiles, snails and insects) non specified [(c)]			
	Fish products [(d)]	LB		0.98
		UB		1.16
	Fish and seafood based meals (composite food) [(e)]			
Water molluscs and Crustaceans	Crustaceans	LB		0.05
		UB		0.20
	Water molluscs	LB		0.01
		UB		0.20

Table D1: Continued.

Ad-hoc food groups for HBCDDs exposure assessment	FoodEx food groups	HBCDD-sum (α+β+γ)		Total HBCDD
		Type	Mean	Mean
Fish offal	Other fish offal (fish liver)	LB		5.10
		UB		5.17
	Fish roe	LB		1.57
		UB		1.57
	Fish offal non specified	LB		3.57
		UB		3.69

LB: lower bound; UB: upper bound.

(a): In the absence of occurrence data for the FoodEx food group (level 2) 'Egg-based meal (composite food e.g., omelette)', LB and UB mean values were assumed to be the same as the ones estimated from the data base on the broad FoodEx food group (level 1) 'Eggs and egg products'.

(b): In the absence of occurrence data for the FoodEx food group (level 2) 'Meat-based meals (composite food)', LB and UB mean values were assumed to be the same as the ones estimated from the data base on the broad FoodEx food group (level 1) 'Meat and meat products'.

(c): In the absence of occurrence data matching with the consumption data at the first level of the FoodEx in the case of 'Fish and other seafood (including amphibians, reptiles, snails and insects)' non specified, the LB and UB mean values from the broad food group of 'Fish and other seafood (including amphibians, reptiles, snails and insects)' were used from Table 5.

(d): In the absence of occurrence data for the FoodEx food group (level 2) 'Fish products' (only one samples in the data base), LB and UB mean values were assumed to be the same as the ones estimated from the data base on the broad FoodEx food group (level 1) 'Fish and other seafood (including amphibians, reptiles, snails and insects)' from Table 5.

(e): In the absence of occurrence data for the FoodEx food group (level 2) 'Fish and seafood based meals (composite food)', LB and UB mean values were assumed to be the same as the ones estimated from the data base on the broad FoodEx food group (level 1) 'Fish and other seafood (including amphibians, reptiles, snails and insects)' from Table 5.

Table D2: Statistical description of concentrations of α-, β- and γ-HBCDD individual stereoisomers, total HBCDD and the sum of the individual stereoisomers (α+β+γ) (number of analysed samples 'n', mean 'MEAN' and percentage of non-detects 'ND %'), calculated on 1,725 samples across the food categories defined by Commission Regulation (EC) No 1881/2006[7], Annex, Section 5. HBCDDs levels (mean concentration) are reported on fat (ng/g fat) or wet weight (ng/g w.w.) basis according to the different food categories as requested by the above mentioned legislation. The mean fat content calculated from the original samples is also reported (%).

Food categories		α-HBCDD			β-HBCDD			γ-HBCDD			HBCDD-sum (α+β+γ)			Total- HBCDDs			Mean percentage of fat in the original sample
	TYPE	n	MEAN	ND (%)[a]	n	MEAN	ND (%)[a]	n	MEAN	ND (%)[a]	n	MEAN	ND (%)[a]	n	MEAN	ND (%)[a]	
Meat and meat products ruminants-ng/g fat	LB	22	0.04	50 %	22	0.01	86 %	22	0.01	95 %	22	0.06					4.44
	UB	22	0.32	50 %	22	0.20	86 %	22	0.29	95 %	22	0.81					4.44
Meat and meat products poultry-ng/g fat	LB	23	0.12	39 %	23	0.03	78 %	23	0.02	87 %	23	0.17					3.60
	UB	23	0.19	39 %	23	0.09	78 %	23	0.28	87 %	23	0.56					3.60
Meat and meat products pigs-ng/g fat	LB	18	0.20	44 %	18	0.00	94 %	18	0.00	100 %	18	0.20					4.50
	UB	18	0.39	44 %	18	0.12	94 %	18	0.29	100 %	18	0.80					4.50
Liver and products terrestrial animals-ng/g fat	LB	25	0.05	92 %	25	0.00	96 %	25	0.01	96 %	25	0.07		7	0.00	100 %	5.81
	UB	25	0.53	92 %	25	0.62	96 %	25	0.45	96 %	25	1.60		7	0.38	100 %	5.81
Muscle meat fish and fish products excl. eel-ng/g w.w.	LB	422	0.34	76 %	422	0.02	91 %	422	0.04	86 %	422	0.39		715	0.42	68 %	5.84
	UB	422	0.83	76 %	422	0.52	91 %	422	0.53	86 %	422	1.88		715	0.62	68 %	5.84
Muscle meat eel-ng/g w.w.	LB	6	6.30	17 %	6	0.17	50 %	6	0.31	50 %	6	6.78		9	0.77	0 %	24.51
	UB	6	6.31	17 %	6	0.18	50 %	6	0.31	50 %	6	6.80		9	0.77	0 %	24.51
Raw milk and dairy products incl. butter-ng/g fat	LB	76	0.03	74 %	76	0.00	74 %	76	0.00	96 %	76	0.03		9	0.02	89 %	5.64
	UB	76	0.23	74 %	76	0.16	74 %	76	0.29	96 %	76	0.67		9	0.15	89 %	5.64
Hen eggs and egg products-ng/g fat	LB	31	0.17	23 %	31	0.00	71 %	31	0.02	77 %	31	0.19		31	0.15	45 %	13.13
	UB	31	0.18	23 %	31	0.02	71 %	31	0.16	77 %	31	0.36		31	0.20	45 %	13.13
Fish liver and products-ng/g w.w.	LB	41	2.59	15 %	41	0.00	100 %	41	0.00	100 %	41	2.59		156	3.63	34 %	10.10
	UB	41	2.74	15 %	41	0.98	100 %	41	0.98	100 %	41	4.69		156	3.74	34 %	10.10
Other products-ng/g w.w.	LB	201	0.08	65 %	201	0.01	89 %	201	0.01	91 %	201	0.09		14	0.47	43 %	11.45
	UB	201	0.15	65 %	201	0.07	89 %	201	0.07	91 %	201	0.28		14	0.56	43 %	11.45
Infant and baby food-ng/g w.w.	LB													16	0.01	69 %	7.24
	UB													16	0.03	69 %	7.24

LB: lower bound; UP: upper bound.
(a): The column ND (%) indicates the percentage of results below the LOD or the LOQ.

Table D3: Percentiles values (90th, 95th and 99th percentiles) of concentrations of α-, β- and γ-HBCDD individual stereoisomers, total HBCDD and the sum of the individual stereoisomers (α+β+γ) calculated on 1,725 samples across the food categories defined by Commission Regulation (EC) No 1881/2006,[7] Annex, Section 5.

		α-HBCDD				β-HBCDD				γ-HBCDD				HBCDD-sum (α+β+γ)				Total- HBCDDs			
Food categories	**TYPE**	**n**	**P90**	**P95**	**P99**	**n**	**P90**	**P95**	**P99**	**n**	**P90**	**P95**	**P99**	**n**	**P90**	**P95**	**P99**	**n**	**P90**	**P95**	**P99**
Meat and meat products ruminants-ng/g fat	LB	22	0.14	0.20	0.36	22	0.02	0.03	0.10	22	0.00	0.00	0.28	22	0.16	0.23	0.74				
	UB	22	0.50	1.06	3.57	22	0.35	1.42	1.59	22	0.48	0.50	1.59	22	1.09	2.84	6.75				
Meat and meat products poultry-ng/g fat	LB	23	0.25	0.55	1.17	23	0.05	0.06	0.58	23	0.07	0.18	0.19	23	0.48	0.79	1.75				
	UB	23	0.49	0.55	1.17	23	0.13	0.49	0.58	23	0.58	0.91	0.94	23	1.29	1.31	2.34				
Meat and meat products pigs-ng/g fat	LB	18	0.33	2.48	2.48	18	0.00	0.01	0.01	18	0.00	0.00	0.00	18	0.33	2.48	2.48				
	UB	18	2.48	2.54	2.54	18	0.30	1.13	1.13	18	1.13	1.24	1.24	18	2.97	4.79	4.79				
Liver and products terrestrial animals-ng/g fat	LB	25	0.00	0.00	1.31	25	0.00	0.00	0.02	25	0.00	0.00	0.32	25	0.00	0.34	1.31	7	0.00	0.00	0.00
	UB	25	1.30	1.31	1.75	25	1.21	1.60	2.50	25	0.81	1.10	3.80	25	3.21	3.81	6.70	7	0.41	0.41	0.41
Muscle meat fish and fish products excl. eel-ng/g w.w.	LB	422	0.39	0.98	6.52	422	0.00	0.09	0.39	422	0.04	0.10	1.20	422	0.54	1.30	6.61	715	1.53	2.17	3.93
	UB	422	1.00	1.00	6.52	422	1.00	1.00	1.00	422	1.00	1.00	1.20	422	3.00	3.00	6.61	715	1.53	2.17	3.93
Muscle meat eel-ng/g w.w.	LB	6	25.10	25.10	25.10	6	0.86	0.86	0.86	6	1.67	1.67	1.67	6	27.63	27.63	27.63	9	1.63	1.63	1.63
	UB	6	25.10	25.10	25.10	6	0.86	0.86	0.86	6	1.67	1.67	1.67	6	27.63	27.63	27.63	9	1.63	1.63	1.63
Raw milk and dairy products incl. butter-ng/g fat	LB	76	0.04	0.22	0.55	76	0.01	0.02	0.06	76	0.00	0.00	0.01	76	0.06	0.22	0.55	9	0.20	0.20	0.20
	UB	76	0.48	0.56	0.82	76	0.33	0.46	0.63	76	0.62	0.67	1.10	76	1.44	1.52	2.32	9	0.20	0.20	0.20
Hen eggs and egg products-ng/g fat	LB	31	0.63	0.94	1.33	31	0.01	0.02	0.04	31	0.08	0.10	0.12	31	0.63	1.05	1.43	31	0.32	0.46	0.83
	UB	31	0.63	0.94	1.33	31	0.03	0.03	0.04	31	0.24	0.24	0.63	31	0.90	1.13	1.43	31	0.32	0.46	0.83
Fish liver and products-ng/g w.w.	LB	41	5.00	5.40	10.40	41	0.00	0.00	0.00	41	0.00	0.00	0.00	41	5.00	5.40	10.40	156	9.90	12.66	27.95
	UB	41	5.00	5.40	10.40	41	1.00	1.00	1.00	41	1.00	1.00	1.00	41	7.00	7.40	12.40	156	9.90	12.66	27.95
Other products-ng/g w.w.	LB	201	0.03	0.16	2.49	201	0.00	0.00	0.31	201	0.00	0.00	0.15	201	0.05	0.34	2.63	14	2.55	2.93	2.93
	UB	201	0.42	1.00	2.49	201	0.17	0.31	1.00	201	0.09	0.36	1.00	201	0.77	2.63	3.00	14	2.55	2.93	2.93
Infant and baby food-ng/g w.w.	LB																	16	0.05	0.05	0.05
	UB																	16	0.05	0.05	0.05

n: number of samples; LB: lower bound; UP: upper bound.

E. BODY BURDEN AS DOSE METRIC FOR HBCDD TOXICITY

The body burden (BB, amount/kg b.w.) reflects the accumulation of a chemical at the level of the total body. Assuming toxicity in the i^{th} organ to be directly related to a chemical's organ concentration the time course of the organ concentration $C_i(t)$ can be related to the $BB_{(t)}$, with 't' being the time since the start of exposure to the chemical. The most straightforward way of modeling is to relate $C_i(t)$ to the concentration in blood $C_{bl}(t)$ by means of a partition coefficient p_i:

$$C_i(t) = p_i \cdot C_{bl}(t) \quad (1)$$

with:

p_i the organ-blood partition coefficient
$C_{bl}(t)$ the concentration in the blood (amount/kg)

$C_i(t)$ can also be expressed using the total amount of the chemical in a specific organ and the organ volume:

$$C_i(t) = \frac{A_i(t)}{V_i} \quad (2)$$

with:

$A(t)$ total amount in the organ (amount)
Vi organ volume (l)

Consequently $BB_{(t)}$ it is:

$$BB(t) = \frac{\sum_i A_i(t)}{\sum_i V_i} \quad (3)$$

Substituting (1) and (2) into (3) then gives:

$$BB(t) = C_{bl}(t) \cdot \frac{\sum_i p_i V_i}{\sum_i V_i} \quad (4)$$

Denoting the ratio $\frac{V_i}{\sum_i V_i}$ as f_i , i.e. the fraction of the i^{th} organ compartment of the total body weight, in equation (4) then relates *BB(t)*, via $C_{bl}(t)$ and f_i , to the organ specific exposure $p_i C_{bl}(t)$:

$$BB(t) = C_{bl}(t) \cdot \sum_i p_i f_i \tag{5}$$

Basically, equation (5) states that *BB(t)* can be obtained by multiplying the concentration in blood with the organ specific exposure, determined by the product of an organ's affinity for a chemical relative to the blood (as reflected by the organ's partition coefficient p_i) and the organ's fraction of the total body weight (as reflected by f_i). Consequently, the concentration in the blood is determined by the combined effect of these two parameters over all *i* organs.

Regarding HBCDDs, the partition coefficients p_i are in general determined by the organ lipid content. Assuming this content to be constant within a species the partition coefficients likely have a constant value too, with interspecies differences being caused by differences in organ lipid content. An exception may be the partition coefficient p_l in the rodent liver. Whether or not lipid partitioning suffices as the starting point for the *BB(t)* as the dose metric for HBCDD organ concentration, and induced organ toxicity, depends on the possible sequestration of HBCDDs in the liver. In the study by van de Ven et al. (2006) it has been shown that HBCDDs can be found in the liver lipid fraction, but also that concentrations in adipose tissue are higher. Therefore equation (5) is considered valid in relating the *BB(t)* to organ exposure after repeated exposure. It should be noted that in the statistical analysis, interspecies differences in p_i en f_i are considered as 'residual uncertainty' when using the *BB(t)* as dose metric for organ exposure.

The adipose tissue concentration as dose metric for extrahepatic toxicity

In practice, due to the absence of suitable information on the organ specific concentrations as calculated from the *BB(t)* may be available only for a limited number of organs, whereas toxicity data may be available for more organs. In these cases the adipose tissue concentration instead of the BB can be used as a dose surrogate for the concentration in the extrahepatic organs, in particular when the BB does not reflect hepatic sequestration. In the case of thyroid toxicity, for instance, the adipose tissue concentration can be used as a dose surrogate for the thyroid concentration. Assuming (for the sake of simplicity) 'steady state' conditions with respect to HBCDD exposure and thyroid toxicity (E_{thy}) to be induced this toxicity depends on the total 'steady state' thyroid concentration ($C_{thy,ss}$), or:

$$E_{thy} = f(C_{thy,ss}) \tag{6}$$

where *f* denotes the relation (function) in a generic manner.

Furthermore, assuming $C_{thy.ss}$ to be a function of the total 'steady state' adipose tissue concentration $C_{f.ss}$, the following equation holds:

$$C_{thy,ss} = f'(C_{f,ss}) \tag{7}$$

and thus:

$$E_{thy} = f(f'(C_{f,ss})) \tag{8}$$

Assuming the lipid content of the thyroid and the adipose tissue to be constant the 'steady state' distribution between these organs is characterised by the ratio of their lipid partition coefficients the following relationship holds:

$$\frac{C_{thy,ss}}{C_{f,ss}} = \frac{p_{thy}}{p_f} \tag{9}$$

So, in the case of a linear relationship between thyroid toxicity and the total thyroid concentration the induction of thyroid toxicity (E_{thy}) can be expressed as a linear function of the concentration in the adipose tissue:

$$E_{thy} = \alpha \cdot \frac{p_{thy}}{p_f} \cdot C_{f,ss} \tag{10}$$

A more complicated situation occurs when the relationship between thyroid toxicity and the thyroid concentration is non-linear. In such a case a convenient, non-linear dose response relation would be of the form:

$$E_{thy} = a \cdot [c - (c-1) \cdot \exp(-b \cdot C_{thy,ss})] \tag{11}$$

which then leads to the following relation between induced thyroid toxicity and the concentration in the adipose tissue:

$$E_{thy} = a \cdot [c - (c-1) \cdot \exp(-b \cdot \frac{p_{thy}}{p_f} C_{f,ss})] \tag{12}$$

F. DOSE RESPONSE MODELLING

HBCDDs are characterised by their slow removal from the body and, consequently, they are accumulating after repeated exposure. As a result the appropriate dose metric associated with the risk of HBCDDs is the accumulated amount in the body, rather than the daily (external) exposure. The body burden (BB), the total amount in the body divided by body weight, provides a generic dose metric for chemicals accumulating in the body. The BB was used as the dose metric in the risk characterisation of HBCDDs as follows:

Step 1: The determination of the acute/chronic BMDL in the animal and its corresponding BB in the average animal (*BMDL* and BB_a, dimension: amount/kg b.w.).

In the case of a single oral dose the BB_a is:

$$BB_a = F_{abs,a} \cdot BMDL \quad (1)$$

with:

$F_{abs,a}$	Fraction of the chemical in food which is absorbed into the animal body (dimensionless)
BMDL	Bench Mark Dose Lower Limit for animal toxicity (amount/kg b.w.)
BB_a	Body burden in the experimental animal at the BMDL (amount/kg b.w.)

Step 2: The interspecies extrapolation of the BMDL BB in the animal to man. Assuming that exposure to HBCDDs occurs mainly via the food, the chronic human dietary exposure ($D_{r,h}$) is calculated, which leads to this BB in the (average) human corresponding to the BB at the BMDL in animals. As HBCDDs are expected to distribute particularly in the adipose tissue in humans, one compartmental modeling may suffice here. Assuming the chronic human dietary exposure to lead to a 'steady state' situation, $D_{r,h}$ can be calculated by:

$$D_{r,h} = \frac{BB_a \cdot k_{el,h}}{F_{abs,h}} \quad (2)$$

with:

BB_a	body burden in the experimental animal at the BMDL (amount/kg b.w.)
$k_{el,h}$	rate constant[18] for the removal from the human body (dimension: day^{-1})
$F_{abs,h}$	fraction of the chemical in food which is absorbed into the body (dimensionless)
$D_{r,h}$	chronic human daily dietary intake (amount/kg b.w. per day)

The calculated human dietary intake, i.e. $D_{r,h}$ can then be compared with the estimated human dietary intake (see Chapter 9).

BMD analysis

The dose-response analysis and the calculation of the BMD and the BMDL, i.e. its 95 % lower confidence limit was performed using the PROAST software (Slob, 2002). This results in a BMD and its associated uncertainty distribution (confidence interval, CI), related to a predefined benchmark response (BMR). Two families of nested dose response models, the Exponential and Hill models, present in PROAST were fitted to the (continuous) toxicity data. This procedure accounts for the

[18] Note that for one-compartmental modelling the relationship $\ln 2 = k_{el} \cdot t_{1/2}$ holds, with $t_{1/2}$ the half-life in the body, i.e. the time needed for half of the amount of a chemical to be removed from the body once the exposure has stopped.

incorporation of model uncertainty in the dose-response analysis. For the evaluated endpoints both model families resulted in acceptable fits (based on the log-likelihood criteria, see Slob, 2002). The bootstrap technique (1000 runs) was used to generate a BMD distribution for each model (Moerbeek et al., 2004). Both BMD distributions were subsequently combined to generate an overall BMD distribution. In the applied probabilistic risk assessment approach the whole uncertainty distribution around the BMD is used as an input (Bokkers, 2009; Slob and Pieters, 1998).

The quality of the dose-response data was checked by applying the criteria for the application of dose - response modeling in risk characterisation as developed by EFSA (2009). Dose-response data are considered poor, and therefore not informative, when one (or more) of the following criteria are met:

1. the confidence interval around the BMD is wide
2. different models result in widely different BMDL values
3. the BMD is estimated by extrapolation outside the range of observation, such that the BMD(L) would then depend heavily on the model used.

Criteria to judge the adequacy of the dose-response data on the basis of the range of BMDL values obtained (criterion no. 2) have so far not been established. EFSA (2009) proposes that, as a general rule, dose-response data should not result in a range of BMDL values from different accepted models that substantially exceed one order of magnitude. The other two criteria are not quantified either. For consistency reasons, we propose that criteria no. 1 and 3 should meet this requirement too. Thus, the difference between the upper and lower limits of the 90 % CI should not exceed one order of magnitude. Furthermore, the BMD should not be 10 times higher than the highest applied dose level, or 10 times lower than the lowest applied dose level.

Selected toxicity study

The study of Eriksson et al. (2006) identified neurodevelopmental effects on behaviour in neonatal male mice as a sensitive toxic effect of technical HBCDD. In this study neonatal mice were exposed by gavage to a single dose (0, 0.9 and 13.5 mg/kg b.w.) at PND10. At the age of 3 months the mice were tested on their spontaneous motor behavior and habituation capability, i.e. the ability to explore a new environment (total activity, locomotion, rearing).

Using equation (2) for the extrapolation of the BB at PND10 to man basically assumes that in humans this BB is reached after prolonged exposure, i.e. after a time period long enough to reach a 'steady state'. Given an expected human half-life of HBCDDs of 219 (see Chapter 8.1) such a situation may be expected around 2.5-3 years. Assuming the sensitive time window for the induction of neurodevelopmental effects in humans to lie before this age, the application of a 'steady state' BB as a dose metric for the induction of this effect on the period between birth and 2.5-3 years of age might overestimate the risk. Using a 'steady state' BB for the calculation of $D_{r,h}$ guarantees that up to the age of 2.5-3 years the human BB will stay below the BB in mice, which is associated with neurodevelopmental effects. In case sensitivity for neurodevelopmental effects even extends beyond this age, the calculated $D_{r,h}$ guarantees that even during adolescence the human BB will stay just at the level of the BB in mice, which is associated with the onset of neurodevelopmental effects on behaviour.

For the analysis of the data of the study of Eriksson et al. (2006) Figure 1 has been scanned to transform the results into numerical values to be used in the BMD analysis. Spontaneous behaviour (locomotion, rearing and total activity) was tested during three time periods (0-20, 20-40 and 40-60 min) after placing the mice in a new environment. Only the results of the 0-20 min observation period were used, because the dose response for the other periods was poor. The numerical values for the different parameters in this observation period resulting from the graphical transformation are presented in Table F1. The CONTAM Panel selected locomotion and total activity as more reliable parameters than rearing for the BMD analysis.

Table F1: Transformed numerical results for neurodevelopmental effects of HBCDDs on behaviour in mice as derived from Figure 1 of Eriksson et al. (2006).

Dose[(a)] (mg/kg b.w.)	Locomotion (mean ± SD)	Rearing (mean ± SD)	Total activity (mean ± SD)
0	500 ± 83	1,580 ± 280	4,720 ± 580
0.9	415 ± 53	1,190 ± 250	4,460 ± 540
13.5	215 ± 59	282 ± 77	2,480 ± 330

b.w.: body weight; SD: standard deviation.
(a): N=10.

The nested character of the family of models (exponential or Hill models) makes it possible to formally choose a model for describing a particular data set. In general, when a model is extended by one or more parameters the resulting fit criterion may achieve a higher value compared to the model with fewer parameters. However, it is unfavourable to use a model with too many parameters, as this results in reduced precision of model predictions. Therefore, a formal criterion is needed to decide whether extension in the number of parameters should be accepted or not. For nested models this is done by the likelihood ratio test. A formal decision criterion using this test is the 5 % significance level. In the PROAST software used by the CONTAM Panel for the BMD analysis the appropriate model is automatically selected by consecutively fitting the members of the model family and choosing the model that cannot be significantly improved by a model having more parameters, as determined by the likelihood ratio test (Slob, 2002).

The results of the BMD analysis are presented in Table F2 and the respective dose response curves in Figures F1 and F2. The CONTAM Panel chose a benchmark response (BMR) of 10 % to avoid extrapolation beyond the observable range (EFSA, 2009). The lowest BMD and BMDL are found for the parameter locomotion. Therefore the BMD of 1.16 mg/kg b.w. and its $BMDL_{10}$ of 0.93 mg/kg b.w. are the endpoints to be used in the hazard assessment of HBCDDs.

Table F2: Overview of BMDs and 90 % CIs (benchmark dose lower limit: BMDL, benchmark dose upper limit: BMDU) for neurodevelopmental effects of HBCDDs on behaviour in mice (Eriksson et al., 2006). BMR: 10 %.

Endpoint (observation period)	Model [(a)]	Log likelihood	BMD mg/kg b.w.	90 % CI	
				BMDL	BMDU
Locomotion[(b)] (0-20 min)	E	6.69	1.76	1.51	2.09
	H	7.12	1.16	0.93	1.49
Rearing (0-20 min)	E	2.63	0.85	0.78	0.04
	H	4.33	0.32	0.26	0.38
Total activity[(b)] (0-20 min)	E	21.29	2.23	1.98	2.55
	H	21.33	1.65	1.40	1.98

b.w.: body weight; BMR: benchmark response; CI: confidence interval.
(a): E = exponential model, H = Hill model.
(b): See Figure G1and G2 for BMD analysis.

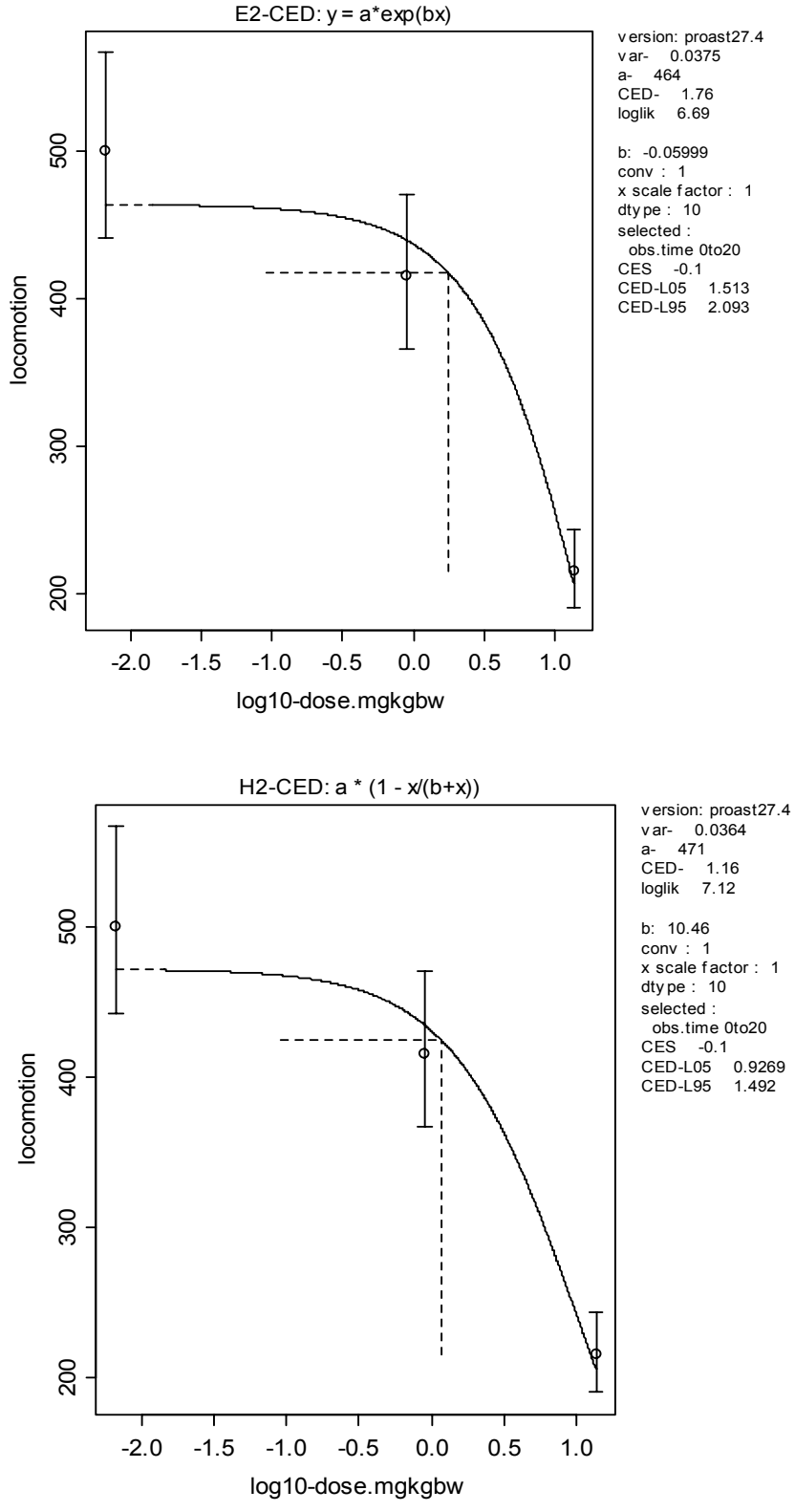

Figure F1: Dose-response analysis of locomotion (counts) against the HBCDD dose for the 0-20 minute observation period in 3 month old mice (Eriksson et al., 2006). Model fit: Hill (upper) and Exponential (lower), BMR: 10 %.

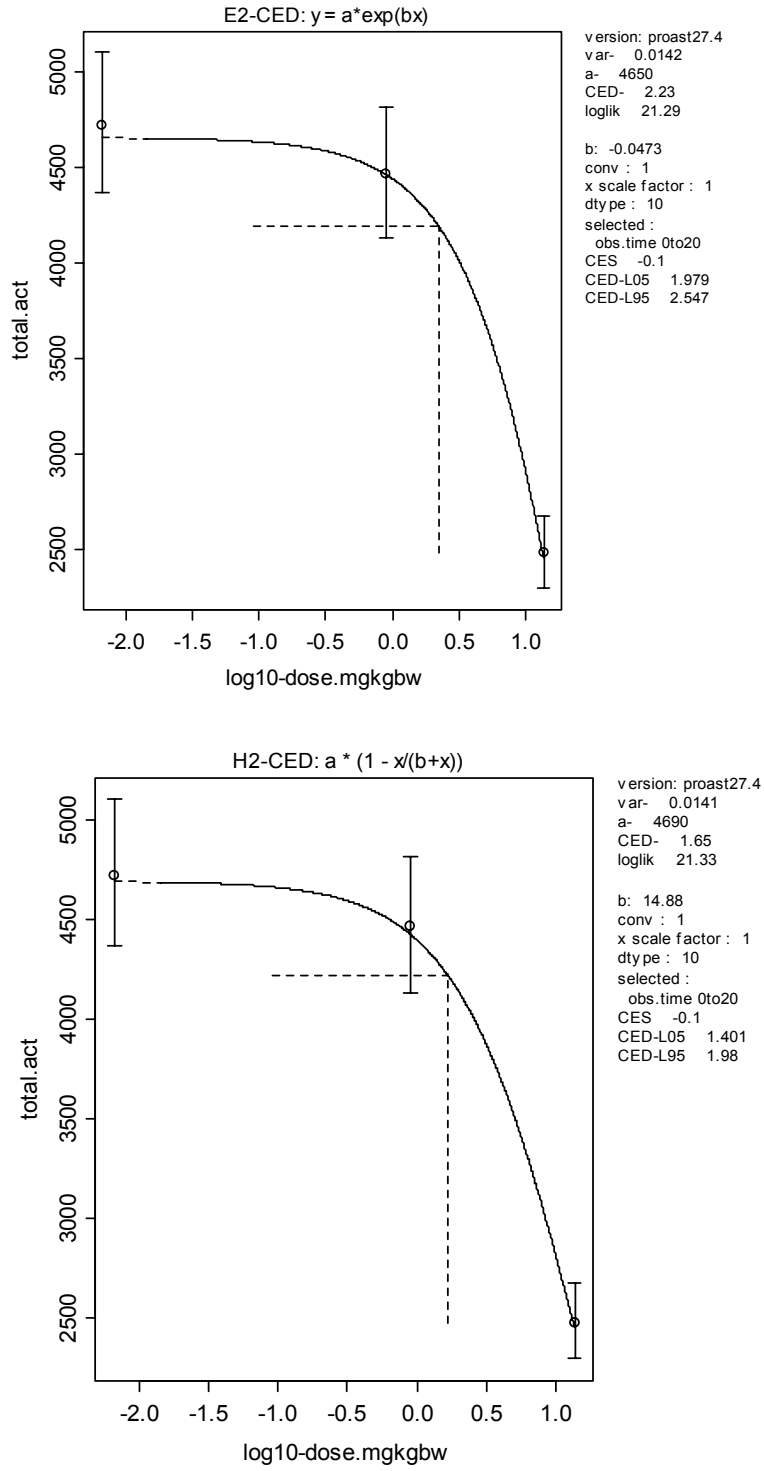

Figure F2**:** Dose-response analysis total activity against the HBCDD dose for the 0-20 minute observation period in 3 month old mice (Eriksson et al., 2006). Model fit: Hill (upper) and Exponential (lower), BMR: 10 %.

ABBREVIATIONS

AESAN	Agencia Española de Seguridad Alimentaria y Nutricion
AhR	Aryl hydrocarbon receptor
APCI	Atmospheric pressure chemical ionisation
BAEP	Brainstem auditory evoked potentials
BB	Body burden
BCF	Bioconcentration factor
BE	Belgium
BFR	Brominated flame retardant
BG	Bulgaria
BMD	Benchmark dose
BMDL	Benchmark dose lower confidence limit; 95 %-confidence lower bound
BMDU	Benchmark dose – upper limit
BMFs	Biomagnification factors
BMR	Benchmark response
BTBPE	Bis(2,4,6-tribromophenoxy)ethane
b.w.	Body weight
CAR	Constitutive androstane receptor
CH	Switzerland
CI	Confidence interval
CONTAM Panel	Panel on Contaminants in the Food Chain
Comprehensive Database	Comprehensive European Food Consumption Database
CRM	Certified Reference Material
CY	Cyprus
CZ	Czech Republic
DATEX	Data Collection and Exposure Unit (EFSA), currently DCM Unit (EFSA)
DBDPE	Decabromodiphenyl ethane
DCM	Dietary and Chemical Monitoring Unit (EFSA), former DATEX Unit (EFSA)
DE	Germany
DK	Denmark
DMSO	Dimethylsulfoxide
d.w.	Dry weight
DXA	Dual energy X-ray absorptiometry
EBFRIP	European Brominated Flame Retardants Industry
EC	European Commission
EC_{50}	Half maximal effective concentration
ECB	European Chemicals Bureau
ECNI	Electron chemical negative ionization
EF	Enentiomer fraction
EFSA	European Food Safety Authority
EGTA	Ethylene glycol tetraacetic acid
EI	Electron impact
EPS	Expanded polystyrene
ES	Spain
ESI	Electrospray ionisation
EU	European Union
EU27	The 27 European Union Member States
EXPOCHI	Article 36 project 'Individual food consumption data and exposure assessment studies for children'
FI	Finland

FR	France
GC	Gas chromatography
GC-HRMS	Gas chromatography-high resolution mass spectrometry
GD	Gestational day
GEMS/Food	Global Environment Monitoring System-Food Contamination Monitoring and Assessment Programme
GPC	Gel permeation chromatography
GR	Greece
HBCDDs	Hexabromocyclododecanes
HeLa	Human cervical cancer cells
HIPS	High impact polystyrene
HPLC-MS/MS	High-performance liquid chromatography – tandem mass spectrometry
HU	Hungary
HUMIS	Norwegian Human Milk Study
IC_{50}	Half maximal inhibitory concentration
IE	Ireland
IgG	Immunoglubulin A
IOM	Institute of Medicine of the United States National Academies of Sciences
IT	Italy
ITMS	Ion trap-mass spectrometry
IUPAC	International Union of Pure and Applied Chemistry
LB	Lower bound
LC	Liquid chromatography
LOD	Limit of detection
LOEL	Lowest-observed-effect level
Log K_{ow}	Octanol-water partitioning coefficient
LOQ	Limit of quantification
LRMS	Low-resolution mass spectrometry
LRTAP-POPs	Long-Range Transboundary Air Pollution on Persistent Organic Pollutants
LT	Latvia
ML	Maximum level
MOE	Margins of exposure
MOSs	Margins of safety
N	Frequency of results
ND	Not detected
NL	The Netherlands
NO	Norway
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
PBB	Polybrominated biphenyls
PBDD/Fs	Polybrominated dibenzo-*p*-dioxins and furans
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PLE	Pressurised liquid extraction
PND	Postnatal day
PNW	postnatal week
POPRC	The Persistent Organic Pollutants Review Committee
POP	Persistent Organic Pollutants
PTV	Programmable temperature vaporisation injector
PXR	Pregnane X receptor
QA	Quality assurance

QC	Quality control
SRBC	Sheep red blood cells
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
ROS	Reactive oxygen species
RSD	Relative standard deviation
RSV	Respiratory syncytial virus
SE	Sweden
SPE	Solid-phase extraction
SRM	Standard reference materials
T3	Triiodothyronine
T4	Thyroxine
TBBPA	Tetrabromobisphenol A
TDS	Total diet study
TSH	Thyroid stimulating hormone
TTR	Transthyretin
UB	Upper bound
UGT	UDP glucuronosyltransferase
UK	United Kingdom
UV	Ultraviolet
VN	Vietnam
w.w.	Wet weight
XPS	Extruded polystyrene