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Update of the risk assessment of hexabromocyclododecanes (HBCDDs) in food

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Abstract

The European Commission asked EFSA to update its 2011 risk assessment on hexabromocyclododecanes (HBCDDs) in food. HBCDDs, predominantly mixtures of the stereoisomers α-, β- and γ-HBCDD, were widely used additive flame retardants. Concern has been raised because of the occurrence of HBCDDs in the environment, food and in humans. Main targets for toxicity are neurodevelopment, the liver, thyroid hormone homeostasis and the reproductive and immune systems. The CONTAM Panel concluded that the neurodevelopmental effects on behaviour in mice can be considered the critical effects. Based on effects on spontaneous behaviour in mice, the Panel identified a lowest observed adverse effect level (LOAEL) of 0.9 mg/kg body weight (bw) as the Reference Point, corresponding to a body burden of 0.75 mg/kg bw. The chronic intake that would lead to the same body burden in humans was calculated to be 2.35 μg/kg bw per day. The derivation of a health-based guidance value (HBGV) was not considered appropriate. Instead, the margin of exposure (MOE) approach was applied to assess possible health concerns. Over 6,000 analytical results for HBCDDs in food were used to estimate the exposure across dietary surveys and age groups of the European population. The most important contributors to the chronic dietary LB exposure to HBCDDs were fish meat, eggs, livestock meat and poultry. The CONTAM Panel concluded that the resulting MOE values support the conclusion that current dietary exposure to HBCDDs across European countries does not raise a health concern. An exception is breastfed infants with high milk consumption, for which the lowest MOE values may raise a health concern.

Keywords: hexabromocyclododecanes, HBCDDs, occurrence, food, toxicology, human exposure, risk assessment

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Summary

Brominated flame retardants (BFRs) are anthropogenic chemicals, which are used in a wide variety of consumer/commercial products in order to improve their resistance to fire. Concern has been raised because of the occurrence of several chemical compounds from the group of BFRs in the environment, food and in humans. This has led to bans on the production and use of certain formulations.

The European Commission asked European Food Safety Authority (EFSA) to update its 2010–2012 risk assessments on the different families of BFRs, i.e. hexabromocyclododecanes (HBCDDs), polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A (TBBPA) and its derivatives, brominated phenols and their derivatives and novel and emerging BFRs.

The similarities in chemical properties and effects seen in the previous EFSA assessments for the different BFR families warrant the consideration of a mixture approach. The Panel on Contaminants in the Food Chain (CONTAM Panel) will evaluate the appropriateness of applying a mixture approach in an additional Opinion once the risk assessment for each BFR family has been updated. It will be based on the EFSA Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals.

This first Opinion updates the risk assessment of HBCDDs in food previously performed by EFSA and published in 2011. The assessment focuses on 1,2,5,6,9,10-HBCDD, which is predominantly composed of the three stereoisomers α-, β- and γ-HBCDD. The Panel developed the draft scientific Opinion which underwent a public consultation from 14 October 2020 to 25 November 2020. The comments received and how they were taken into account when finalising the scientific Opinion are available in Annex E to this scientific Opinion.

HBCDDs are additive flame retardants used mainly in expanded and extruded polystyrene applied as construction and packing material and in textiles. HBCDDs were permitted for general use in the EU until August 2015, after which only authorised applications were allowed because of health concerns.

The present assessment takes into account the occurrence data in food and biological samples, submitted after the publication of the former Opinion, as well as newly available scientific information on hazard identification and characterisation.

The analytical determination of HBCDDs is performed either by gas chromatography (GC)/mass spectrometry (MS) or liquid chromatography (LC)/MS-based methods. Both techniques enable the use of isotope-labelled internal standards, which allow for the correction for losses during extraction and clean-up. While GC/MS-based methods determine Total HBCDDs as they cannot separate the stereoisomers, LC/MS-based methods allow the specific analysis of the individual HBCDD stereoisomers.

Hazard identification and characterisation

In rodents, absorption of HBCDD stereoisomers from the gastrointestinal tract is rapid and almost complete (> 80%). HBCDDs and/or their metabolites are distributed to a number of tissues, including adipose tissue, muscle, liver, skin and brain. Following a single oral administration in rats, α-HBCDD and β-HBCDD accumulate in adipose tissue, while γ-HBCDD accumulates in liver and adipose tissue. In repeated dose experiments in mice, α-HBCDD and β-HBCDD accumulate mainly in adipose tissue, while γ-HBCDD accumulates in the liver. Metabolic debromination and hydroxylation of HBCDDs have been reported. Conversion of γ-HBCDD to α- and β-HBCDD has been reported, but no stereoisomerisation of α-HBCDD. Elimination of HBCDD stereoisomers in rodents is predominantly via faeces. The elimination half-lives of HBCDD stereoisomers in female mice, based on concentrations in adipose tissue, vary from 3 to 4 days for γ-HBCDD, 2.5 days for β-HBCDD and 17 days for α-HBCDD.

In humans, in the only available study, the half-life was estimated to be 64 days (range 23–219 days) for the sum of α-, β- and γ-HBCDD stereoisomers. This difference in kinetics affects the extrapolation of animal data to humans.

Transfer of HBCDDs was studied in different animal species (laying hens, broilers, ducks, pigs and fish). HBCDDs are distributed to a number of tissues and accumulate in tissues with high-fat content and eggs.

The acute toxicity of HBCDDs is low. Toxicological studies have been carried out using different experimental designs with single or repeated administration during gestation, postnatally or in adulthood using, in most cases, HBCDDs with no information on the stereoisomer composition specified. Main targets for HBCDD toxicity are neurodevelopment, the liver, thyroid hormone homoeostasis and the reproductive and immune systems.

HBCDDs induced increased liver weight and hepatocellular hypertrophy in rats and mice. Increased thyroid weight, follicular hypertrophy, hyperplasia and/or colloid depletion and changes in thyroid

hormone levels were also reported in these species. HBCDDs decreased the fertility index and increased pup mortality during lactation in a two-generation toxicity study in rats. In addition, HBCDDs decreased testes weight and delayed vaginal opening in female pups in a one-generation study in rats. Other endocrine-related effects were changes in levels of sex steroid hormones (testosterone and oestradiol) in mice, decreased number of primordial ovarian follicles or reduction in the number of growing follicles in rats.

HBCDDs affected the immune system. Changes observed included decreased thymus weight and increased lesions in the thymus, reduced splenocytes proliferation and increased spleen lesions, decreased T-cell and increased B-cell populations, increased natural killer (NK) cells and changes in immunoglobulin responses.

Exposure of juvenile rats and mice to HBCDDs caused neurodevelopmental effects on behaviour, leading to changes in spontaneous behaviour, spatial learning and memory as detected in adulthood.

HBCDDs are not genotoxic *in vitro* or *in vivo*. The slight induction of DNA strand breaks observed in some *in vitro* tests is most likely due to oxidative stress.

Based on the available information to the CONTAM Panel, i.e. the lack of carcinogenicity in the mouse study, the lack of direct genotoxicity and the information available on mode of action, there is no indication that HBCDDs are carcinogenic.

A growing number of epidemiological publications were identified assessing the association between exposure to HBCDDs and birth weight/length, neurodevelopment and thyroid dysfunction in children, as well as subfertility, type 2 diabetes, thyroid hormone levels, severe endometriosis (including ovarian endometrioma) and breast cancer metastasis in adults. None of the effects studied in longitudinal studies and using internal exposure measures either reached statistical significance or were replicated in a longer follow-up point in the same study. Considerable limitations exist pertaining to small sample sizes, varying methodological quality, effect inconsistency and considerable heterogeneity in the assessed populations, exposures and endpoints. Adverse effects of HBCDDs related to neurodevelopment have been assessed in two epidemiological studies; the low volume of prospective data, the differing endpoint measures and the lack of replication render these data insufficient for use in risk characterisation.

Regarding the mode of action, effects of HBCDDs on the liver of rodents appear to involve the constitutive androstane receptor (CAR) and the pregnane-X-receptor (PXR). Mitochondrial energy production is reduced with downstream effects on reactive oxygen species (ROS) production, Na^+/K^+-ATPase and Ca^{2+}-ATPase activities and increased cytosolic [Ca^{2+}] and [Zn^{2+}]. HBCDDs stimulate proliferation and migration of liver cell lines at picomolar to nanomolar concentrations and there is evidence that these effects are related to activation of the PI3K/Akt/mTOR and oestrogen receptor signalling pathways. These effects might contribute along with activation of CAR, PXR and PPARs to the observed increase in liver weight in rodent studies with HBCDDs. Oral HBCDD exposure of mice fed a high-fat diet aggravates metabolic dysfunction through modifications of lipid and glucose homoeostasis. HBCDD-induced lipid accumulation in liver and adipose tissue appears to be associated with a suppression of the Wnt/β-catenin pathway resulting in increased expression of *Pparg* and its adipogenic target genes. Mechanistic studies on the effects of HBCDDs on the nervous system support specific effects on dopaminergic and glutamatergic neurons. This appears to include inhibition of dopamine and glutamate reuptake following synaptic release, and deregulation of Ca^{2+} and Zn^{2+}. The brain could also be affected by HBCDDs through diminished responsiveness to thyroid hormones. HBCDDs may affect the synthesis of sex steroid hormones by interfering with cAMP-dependent cholesterol uptake and by causing dysregulation of enzymes involved in sex steroid metabolism. HBCDDs may also alter the response of tissues to sex steroids. The mechanism(s) involved in HBCDD-induced ROS production may be related to impairment of mitochondrial energy production.

The evidence from the available human data was not sufficient to base the risk assessment on. Thus, the data from studies on experimental animals were used to identify a Reference Point for the human health risk characterisation. The CONTAM Panel concluded that the neurodevelopmental effects on behaviour can be considered the critical effect for the risk characterisation. The BMD modelling on the neurobehavioural data showed BMD confidence intervals that were relatively wide and that the BMDLs for horizontal locomotion and total activity were far below the lowest dose administered. Therefore, the no observed adverse effect level (NOAEL)/lowest observed adverse effect level (LOAEL) approach was applied. Based on effects on spontaneous behaviour (horizontal locomotion, rearing and total activity), the CONTAM Panel identified a LOAEL of 0.9 mg/kg bw (single dose) as the Reference Point for the risk assessment of HBCDDs.

Because the elimination kinetics for HBCDDs between mice and humans differ, the CONTAM Panel first calculated a body burden of 0.75 mg/kg bw at the LOAEL, considering an oral absorption of

83% in mice. The chronic intake that would lead to the same body burden in humans was calculated assuming an absorption in humans of 100% and the longest half-life identified in humans for HBCDDs of 219 days. This resulted in an estimated chronic human dietary intake of 2.35 μg/kg bw per day.

Due to limitations in the database on HBCDDs, the derivation of a health-based guidance value (HBGV) was not considered appropriate. Instead, the margin of exposure (MOE) approach was applied to assess possible health concerns. The CONTAM Panel considered that an MOE higher than 24 would indicate a low health concern. This MOE would be sufficient to cover interspecies and intraspecies differences in dynamics (a factor of 2.5 and 3.2, respectively), as well as extrapolation from an LOAEL to a NOAEL (a factor of 3). The Panel considered whether an additional factor should be applied to allow for limitations in the database. It was noted that reproductive toxicity studies, that include a battery of tests for evaluating potential neurotoxicity, showed only sporadic effects. It was also noted that there is no indication that HBCDDs are carcinogenic. Taking all this information into account, it was concluded that an additional uncertainty factor for limitations in the database is not needed.

Occurrence and dietary exposure assessment for the European population

A total of 6,857 analytical results for HBCDDs in food fulfilled the quality criteria applied and from these, 6,352 were selected and used for the chronic exposure assessment. Contrary to the data used in the previous Opinion on HBCDDs published in 2011, where most of the data were analysed with GC/MS and reported Total HBCDDs, the current data have mostly been analysed by LC/MS and reported on the specific stereoisomers α-, β- and γ-HBCDD.

The high proportion of left-censored data, partially due to relatively high limits of quantification (LOQs), resulted in a large difference between the lower bound (LB) and upper bound (UB) occurrence values for many food groups. The highest mean concentrations of HBCDDs (sum of α-, β- and γ-HBCDD) were recorded for the food category 'Fish meat', with the highest mean concentrations in eel, being 2.33 and 2.35 μg/kg ww at LB and UB, respectively.

The mean dietary exposure estimates for HBCDDs ranged from 0.07 (minimum LB)/0.17 (minimum UB) to 0.79 (maximum LB)/1.52 (maximum UB) ng/kg bw per day across dietary surveys and age groups. At the 95th percentile, dietary exposure estimates ranged from 0.23 (minimum LB)/0.45 (minimum UB) to 2.30 (maximum LB)/3.61 (maximum UB) ng/kg bw per day. The high proportion of left-censored data, partially due to relatively high LOQs, resulted in a two- to threefold difference between the LB and UB exposure estimates.

The most important contributors to the chronic dietary LB exposure to HBCDDs were 'Fish meat', 'Eggs, fresh', 'Livestock meat' and 'Poultry'.

An estimation of the exposure to HBCDDs of breastfed infants via consumption of human milk was also performed. The exposure scenario based on average human milk consumption and the reported UB range for HBCDD (sum of α-, β- and γ-HBCDD) in pooled human milk samples collected in European countries between 2014 and 2016 as part of the World Health Organisation/United Nations Environment Programme (WHO/UNEP) field studies would result in a median daily exposure of 14.3 ng/kg bw (range: 3.2–73.7 ng/kg bw). For infants with high human milk consumption, the median daily exposure would result in 21.5 ng/kg bw (range 4.8–110.6 ng/kg bw).

Non-dietary exposure through intake of dust and dermal contact can substantially contribute, and in some cases even dominate the total human exposure to HBCDDs, especially for toddlers.

Risk characterisation

MOE values were calculated by comparison of the calculated chronic human dietary intake of 2.35 μg/kg bw per day, leading to the body burden at the LOAEL, with the estimated dietary exposure for the different population groups. The MOE values obtained ranged from 34,000 to 650. These MOEs are larger than 24 and the CONTAM Panel concluded that they do not raise a health concern.

The CONTAM Panel also compared the body burden of 0.75 mg/kg bw at the LOAEL with the body burdens estimated in adults based on levels in adipose tissue, blood and milk reported in the literature. The results support the conclusion that current dietary exposure to HBCDDs in European countries does not raise a health concern.

For breastfed infants, the lowest MOE values for high milk consumption are below the value of 24. The CONTAM Panel concluded that these MOEs may raise a health concern for some breastfed infants.

Recommendations

In order to improve the risk assessment and reduce the uncertainties, the CONTAM Panel made the following recommendations:

- Criteria for the analysis of HBCDD stereoisomers should be set.
- Surveillance of HBCDD stereoisomers in food, in particular in food groups for infants and small children, should continue in order to refine the exposure estimates.
- More data on occurrence of HBCDDs in human milk are needed to enable a more robust exposure assessment for breastfed infants.
- Improved information on toxicokinetics, e.g. half-life values, of HBCDD stereoisomers in humans is needed. More information is needed on the body burden in mothers and relation to transfer of HBCDDs to milk. This information should be used to develop a toxicokinetic model for HBCDDs, including excretion into breast milk and placental transfer.
- More information is needed on the transfer into ruminant meat and milk.
- Further toxicological studies should be conducted with individual HBCDD stereoisomers most relevant to human exposure.
- Studies on neurodevelopment (after repeated exposure), including investigations of the mechanisms and mode of action involved, are recommended.
- Studies on reproductive effects are recommended.
- Studies on possible diabetogenic and obesogenic effects are recommended.
- Longitudinal epidemiological studies of sufficient power and appropriate exposure and co-exposure assessment are needed.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

Background

Brominated flame retardants (BFRs) are anthropogenic chemicals, which are added to a wide variety of consumer/commercial products in order to improve their fire resistance. The major classes of BFRs are brominated bisphenols, diphenyl ethers, cyclododecanes, phenols, biphenyl derivatives and the emerging and novel BFRs.

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 humans. This has led to bans on the production and use of certain formulations of polybrominated diphenyl ethers (PBDEs).

Between September 2010 and September 2012, the Scientific Panel on Contaminants in the Food of EFSA adopted six scientific Opinions on different classes of brominated flame retardants[1]. Because in its Opinion EFSA highlighted several data gaps, hampering the consumer risk assessment for these substances, by means of Commission Recommendation 2014/118/EU on the monitoring of traces of brominated flame retardants in food, Member States were recommended to collect in 2014 and 2015 occurrence data for specific substances in specific foodstuffs.

The newly available occurrence data would enable an updated consumer exposure assessment. Furthermore, since the publication of the EFSA scientific Opinions between 2010 and 2012, new scientific information has become available, therefore it would be necessary to verify whether an update of these scientific Opinions would be appropriate, including an update of the consumer risk assessment.

Terms of reference

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority for an updated exposure assessment for the brominated flame retardants, covered by Recommendation 2014/118/EU, taking into account the occurrence data in food, submitted after the publication of the 2010–2012 EFSA scientific Opinions, and an updated consumer risk assessment, taking into account newly available scientific information.

1.2. Interpretation of the Terms of Reference

Following the request from the European Commission, the CONTAM Panel will update its 2010–2012 risk assessments on the different families of BFRs: hexabromocyclododecanes (HBCDDs), polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A (TBBPA) and its derivatives, brominated phenols and their derivatives and novel and emerging BFRs (EFSA CONTAM Panel, 2011a,b,c, 2012a,b).

This first Opinion updates the risk assessment of HBCDDs in food previously performed by EFSA (EFSA CONTAM Panel, 2011a). The assessment covers the transfer from feed to food of animal origin as a relevant source of HBCDDs in food. The assessment focuses on 1,2,5,6,9,10-HBCDD and its major constituents, the three stereoisomers α-, β- and γ-HBCDD. The CONTAM Panel noted that in a few publications and dossiers, HBCDDs are also mentioned with the substitution pattern 1,3,5,7,9,11. Considering the manufacturing process for HBCDDs, the formation of a structure described by the name 1,3,5,7,9,11-hexabromocyclododecane is unlikely. No REACH registration has been requested for a product with this substitution pattern. Therefore, 1,3,5,7,9,11-HBCDD is not further considered in this assessment.

The similarities in chemical properties and effects seen in the previous EFSA assessments for the different BFR families warrant the consideration of a mixture approach. The CONTAM Panel will evaluate the appropriateness of applying a mixture approach in an additional Opinion once the risk assessment for the each BFR family has been updated. It will be based on the EFSA Guidance on

[1] EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on Polybrominated Biphenyls (PBBs) in Food. EFSA Journal 2010;8(10):1789, 151 pp. Scientific Opinion on Polybrominated Diphenyl Ethers (PBDEs) in Food. EFSA Journal 2011;9(5):2156, 274 pp. Scientific Opinion on Hexabromocyclododecanes (HBCDDs) in Food. EFSA Journal 2011;9(7):2296, 118 pp. Scientific Opinion on Tetrabromobisphenol A (TBBPA) and its derivatives in food. EFSA Journal 2011;9(12):2477, 61 pp. Scientific Opinion on Brominated Flame Retardants (BFRs) in Food: Brominated Phenols and their Derivatives. EFSA Journal 2012;10(4):2634, 42 pp. Scientific Opinion on Emerging and Novel Brominated Flame Retardants (BFRs) in Food. EFSA Journal 2012;10(10):2908, 125 pp.

harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals (EFSA Scientific Committee, 2019).

1.3. Supporting information for the assessment

1.3.1. Physico-chemical properties

Technical HBCDD is a white odourless solid compound, which is sometimes used in combination with other flame retardants. It is important to note that HBCDDs exist as stereoisomers of the cyclododecane molecule, which is substituted with six bromine atoms as shown in **Figure 1**. The physico-chemical properties of HBCDDs were described in Section 1.3 (Chemical characteristics) of the previous EFSA Opinion on HBCDDs (EFSA CONTAM Panel, 2011a) and are summarised in **Table 1**.

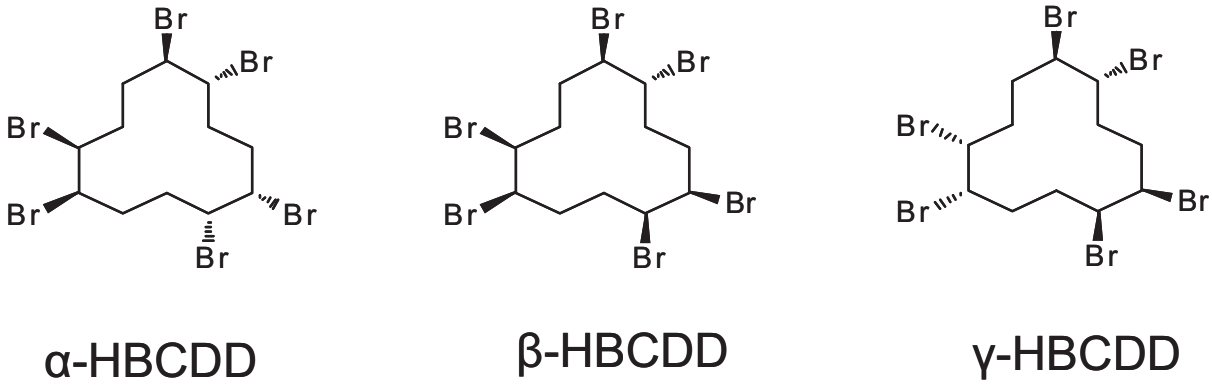

Figure 1: General structure of the three major HBCDD stereoisomers; α-HBCDD, β-HBCDD and γ-HBCDD (molecular mass = 641.7)

Table 1: Physico-chemical properties of HBCDDs (summarised from EFSA, 2011a)

Parameter	α-HBCDD	β-HBCDD	γ-HBCDD
Log Kow (ECB, 2008)	5.07	5.12	5.47
Log Kow (Goss et al., 2008) modelled by COSMOtherm	5.59	5.44	5.53
Water solubility[(a)]	48.8 ± 1.9 μg/L	14.7 ± 0.5 μg/L	2.1 ± 0.2 μg/L

(a): The full set of physico-chemical characteristics is given in the ECB report (ECB, 2008).

Technical products of HBCDDs consist predominantly of the three stereoisomers shown above and contain about 9–13% α-HBCDD, < 0.5–12% β-HBCDD and 72–90% γ-HBCDD (Peled et al., 1995). Other stereoisomers, δ- and ε-HBCDD may be present at low concentrations in the technical product (Law et al., 2005; Arsenault et al., 2007), which may also contain 1–2% tetrabromocyclododecene (Peled et al., 1995). Each of the stereoisomers of HBCDDs has two enantiomers so for the three main stereoisomers, the six enantiomers are (+)-α-HBCDD, (+)-β-HBCDD, (+)-γ-HBCDD, (–)-α-HBCDD, (–)-β-HBCDD and (–)-γ-HBCDD.

HBCDDs are relatively stable, but when they degrade the process primarily consists of the elimination of hydrogen bromide leading to the formation of double bonds in the resulting molecule. The γ-HBCDD stereoisomer can be rearranged to form α-HBCDD at temperatures of 190°C (Peled et al., 1995). Trans-1,2-dibromo-substituted compounds are known to undergo thermal rearrangements (Peled et al., 1995). β-HBCDD is not affected by the thermal rearrangement process (Fång, 2007). Exposure to UV irradiation may lead to breakdown of the parent compound and/or transformation from γ- to α-HBCDD (Harrad et al., 2009), which has important consequences for sampling and analytical considerations (see **Section 1.3.4**). The γ-α transformation contributes to the fact that α-HBCDD is the predominant of the three main HBCDD stereoisomers in the environment.

Differences in solubility between stereoisomers may also have an impact on the changes that occur in ratio of stereoisomers in the environment and food chain. The α-HBCDD stereoisomer predominates in food samples, but it is unclear whether this is a result of a biotransformation process, or whether selective absorption as a result of differences in solubility can also contribute to this change (Zegers et al., 2005; Law et al., 2006) (see **Section 1.3.3**).

1.3.2. Production and industrial use

The processes used to produce HBCDDs were described in Section 4.1 of the previous EFSA Opinion (EFSA CONTAM Panel, 2011a). Some common trade names under which technical HBCDD was marketed, and some details of historic production volumes are also given in the same section in EFSA CONTAM Panel (2011a).

Prior to concerns about toxicity and environmental safety, HBCDDs were a high production volume chemical. In February 2011, HBCDDs were listed in Annex XIV of REACH regulations, and as such became subject to authorisation. Their use has been permitted until the 'sunset date' of August 2015, after which only authorised applications were allowed in the EU.[2] In May 2013, the Stockholm Convention on Persistent Organic Pollutants (POPs) decided to include HBCDDs in Annex A of the Convention, which is a list of chemicals whose production and use should be eliminated.[3]

HBCDDs have been used mostly in expanded and extruded polystyrene foams (EPS and XPS) and as insulation material in the construction industry. Because it is so efficient, only low percentages are required to reach desired standards (0.7% in EPS and 2.5% in XPS). HBCDDs have also been used in upholstered furniture, vehicle textiles, car cushions and insulation blocks in commercial haulage vehicles, packaging materials and electrical and electronic equipment (Covaci and Malarvannan et al., 2016).

HBCDDs are not chemically bound to the polymers where found, and consequently are more likely to leach during production, use, disposal and recycling processes.

1.3.3. Environmental levels and fate

HBCDDs are widespread in the global environment. EFSA CONTAM Panel (2011a) described the main sources of release into the environment and these were primarily from the manufacture and use of insulation boards and manufacture and use of textiles. Due to the properties associated with bioaccumulation and persistence, high levels can be found in top predators. Covaci et al. (2006) reported high concentrations in marine mammals and birds of prey and Zacs et al. (2018a) reported high levels in terrestrial wildlife. Reviews showed that the levels of HBCDDs in the environment were generally increasing in all matrices studied and they seemed to correlate with the use of HBCDDs (UNEP, 2010). Use of HBCDDs was expected to decrease since placement in the Stockholm Convention in 2013 (see **Section 1.3.6**) but no conclusive evidence of decreasing levels in the environment has been shown in the scientific literature (Bao et al., 2015; Cao et al., 2018). There are indications from some studies that concentrations in environmental samples are decreasing, but this is not consistent between studies and is not a statistically robust conclusion. This may be because HBCDDs are still present in many items that are in use, and because of the time lag that is needed before decreasing temporal trends can be verified as a result of the restrictions that have been put in place.

The paragraphs below, which do not claim to be a comprehensive review of the literature, give an overview of some aspects related to the environmental fate and levels of HBCDDs.

1.3.3.1. Biodegradation/transformation

Some information on the transformation of HBCDDs in the environment was provided in the previous EFSA Opinion on HBCDDs (EFSA CONTAM Panel, 2011a). As described above in **Section 1.3.1**, α-HBCDD is the most abundant stereoisomer, and this accounts for the predominance of α-HBCDD in the environment, biota and in humans. The dominance of α-HBCDD becomes greater over time and at higher trophic levels.

β- and γ-HBCDD can be bioisomerised into α-HBCDD by organohalide-respiring bacterium *Dehalococcoides mccartyi* strain 195. β-HBCDD was found to transform to α-HBCDD at faster rate than γ-HBCDD, and the enantiomer fractions of (±)α-, (±)β- and (±)γ-HBCDD were fairly constant, indicating a lack of enantioselective biotransformation of these diastereoisomers (Zhong et al., 2018).

Half-lives for the degradation of HBCDDs in water, soil and sediment are shorter than half-lives in fish. This may be seen as counterintuitive especially when the magnitude of the differences is considered, e.g. 35 days in aerobic sediment and 250 days in a 1 kg fish. However, these data are supported by bioaccumulation measurements that demonstrate biomagnification in food webs and low biotransformation rates (ECB, 2008; Arnot et al., 2009).

[2] https://echa.europa.eu/substance-information/-/substanceinfo/100.239.157
[3] www.pops.int

Thermal degradation of HBCDDs results in the formation of high-molecular weight aromatic and polyaromatic brominated decomposition compounds (Dirtu et al., 2014). In the same way as polychlorinated dioxins and furans can be produced when organochlorine compounds are combusted, polybrominated dioxins and furans may be generated in low amounts when organobromine-containing products are combusted (D'Silva et al., 2004; Jogsten et al., 2010; Piskorska-Pliszczynska and Maszewski, 2014; IPEN, 2019). Even a very low level of these brominated dioxins could be significant due to the high toxicity.

It has been reported that pentabromocyclododecanols and isobutoxypentabromocyclododecanes can be formed as environmental transformation products of HBCDDs (possibly formed via hydrolysis in aquatic environments), and they may also be found in technical HBCDD where they are formed as by-products. As a result, they may be found in the environment along with parent HBCDDs (Heeb et al., 2012).

Degradation processes have been reported to be important for HBCDDs (Tomy et al., 2011). Whilst they may be considered persistent in abiotic media, it is not necessarily so in biotic media. This difference in degradation rate taking into account the biotransformation of HBCDDs in biological systems may confound interpretation of temporal trend studies (Tomy et al., 2011).

1.3.3.2. Differences in HBCDD profiles and bioaccumulation

Previous sections have discussed the transformation of HBCDD stereoisomers from γ-HBCDD, which is predominant in technical HBCDD, to α-HBCDD, which is more dominant in the environment and biota, including humans. The ratio of stereoisomers relates to the matrix and the time that has elapsed since the contamination occurred.

HBCDDs have a strong potential to bioaccumulate and biomagnify. They are persistent in the environment and have a potential for long-range environmental transport. The α-HBCDD stereoisomer predominates in food samples, but it is unclear whether this is a result of a biotransformation process, or whether selective absorption can also contribute to this change (Zegers et al., 2005; Law et al., 2006). Differences in solubility between stereoisomers may also have an impact.

One study that suggested that transformation may take place in the plant was a 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, and the uptake of HBCDDs via the roots was determined. The results showed that after 8 weeks, cabbage can contain up to 65 μg/kg in root tissue and 30 μg/kg in shoot tissue. It was also found that the root tissue contained higher levels of γ-HBCDD compared to α- and β-HBCDD in contrast to what is normally observed in most foods. Up to around 40, 18 and 12 μg/kg, respectively, were found in the root issues, whereas in the shoots, the more typical pattern was found, i.e. α-HBCDD was the dominating isomer (18 μg/kg) followed by γ- (8 μg/kg) and β-HBCDD (3 μg/kg).

In a study by Wu et al. (2012), concentrations resulting from uptake were greater in shoots than in roots of maize (Wu et al., 2016a), and accumulation was found to be in the order β-HBCDD > α-HBCDD > γ-HBCDD in roots, and β-HBCDD > γ-HBCDD > α-HBCDD in shoots. Diastereomer- and enantiomer-specific accumulation and biotransformation of HBCDDs in maize (*Zea mays* L.) were investigated by Huang et al. (2016a). The (−)α-, (−)β- and (+)γ-HBCDD enantiomers accumulated to significantly higher levels in maize compared with their corresponding enantiomers. Bioisomerisation from (+)/(−)-β- and γ-HBCDDs to (−)α-HBCDD was frequently observed, and (−)γ-HBCDD was most easily converted, with an efficiency of 90.5 ± 8.2%.

1.3.3.3. Occurrence in the environment

Occurrence in soil

Most data for HBCDDs in soil originate from urban or industrial areas, and show that soil can contain several hundred μg/kg dry weight (dw) (Covaci et al., 2009). In EFSA CONTAM Panel (2011a), the background concentrations reported were < limit of detection (LOD)−6.6 μg/kg dw, median 0.18 μg/kg dw.

Since the publication of the previous Opinion (EFSA CONTAM Panel, 2011a), Cao et al. (2018) reported levels of HBCDD in soils from China. ΣHBCDDs (sum of α-, β- and γ-HBCDD) levels in the surface soils ranged from 0.88 to 6,901 μg/kg dw with a mean value of 122.57 μg/kg dw. HBCDDs in soil close to a manufacturing facility had the highest level (6,901 μg/kg dw). This value was lower than those reported from HBCDD processing plants in Sweden and Belgium/Germany where concentrations of up to 23,200 μg/kg dw were reported (Covaci et al., 2006). It has been shown that there are significant differences among different types of soils (Tang et al., 2014). A study was conducted using

six types of soils including samples taken from waste dumping sites, industrial areas, residential areas, traffic areas, vegetable soils and farmland soils in Ningbo (Zhejiang, China). High ΣHBCDD concentrations were found in the waste dumping sites (60.74 μg/kg dw) and the industrial area (37.9 μg/kg dw), where HBCDDs could originate from local sources (Tang et al., 2014). The mean concentrations of ΣHBCDDs in traffic areas were 31.8 μg/kg dw, which showed higher concentrations than those in residential areas (14.1 μg/kg dw), soils used to grow vegetables (11.0 μg/kg dw) and farmland soils (7.75 μg/kg dw) (Tang et al., 2014). However, the CONTAM Panel noted that it is difficult to compare these values since the depth of the soil from where these samples were taken from is unknown.

Occurrence in sediment

Sediment is frequently monitored for HBCDDs since it is a recognised sink for hydrophobic chemicals. Due to the relatively high organic content in sediments, and emissions to water, it may be expected to find HBCDDs in sediments close to industry and other sources. It should be noted that aerobic degradation is slower than anaerobic and the status of the sediment is an important factor.

Ganci et al. (2019) studied several BFRs, including HBCDDs, in tidal locations of the river Thames in London (UK). ΣHBCDDs (sum of α-, β- and γ-HBCDD) were detected in most samples (91% detection frequency) at an average concentration of 3.7 μg/kg dw (range: < LOD to 38 μg/kg dw). A study on estuarine and marine sediments around the UK reported a comparable range from < LOD to 47.2 μg/kg dw (Barber et al., 2014). Values for lake sediments in the UK ranged from 0.42 to 7.9 μg/kg dw (Yang, 2014; Ganci et al., 2019). The UK has a history of using more BFRs that other European countries, and levels are therefore likely to be higher than from other regions of Europe.

Occurrence in water

HBCDDs are lipophilic and there are little data in the literature for concentrations in water. Most reported data for water are focussed on suspended particles rather than the water itself. Concentrations of HBCDDs in water can range from a few ng/L to a few hundred μg/L. The major fraction of HBCDDs which reaches wastewater treatment plants is eliminated by adsorption to sludge, and only about 20% is released into the environment in effluents (ECB, 2008). HBCDD concentrations in effluents range from about 10 to 30 μg/L (ECB, 2008; Rüdel et al., 2012).

Yang et al. (2019) measured HBCDDs in 107 water samples taken from nine English freshwater lakes from a mix of urban, rural and remote locations between August 2008 and February 2012. Concentrations of HBCDDs declined over the sampling period with half-lives of 5.1 years. Concentrations of HBCDDs ranged from 45 to 890 pg/L and were generally equally distributed between the freely dissolved and particulate phases in the lakewater. Higher concentrations were associated with colder periods at five out of nine English lakes, and γ-HBCDD was consistently the most dominant isomer found. The study found higher levels in the UK compared with North America, reflecting higher per capita use in the UK.

Ruan et al. (2019) investigated the transport of HBCDD enantiomers by microplastics in a wastewater treatment plant as a potential source of exposure. The release of HBCDDs carried by microplastics potentially reached 15.5 g per day, whereas the dissolved HBCDDs in the effluent may reach 0.067 g per day.

Concentrations of HBCDDs in invertebrates and fish can be an indicator of water quality. Data for invertebrates are available for freshwater and marine species and include samples taken from near point sources, from near regional sources and from remote regions. Concentrations range over four orders of magnitude for marine invertebrates as a result of the importance of proximity to sources (ECB, 2008). Environmental monitoring data for fish include samples from both urban/industrial and from remote regions. As for invertebrates, the span of data is large covering five to six orders of magnitude (ECB, 2008).

Occurrence in air and dust

Levels of HBCDDs in air and dust were summarised in the previous Opinion (EFSA CONTAM Panel, 2011a).

In air, HBCDDs are present mainly associated with particulates. Yu et al. (2008) showed concentrations in Guangxhou (China) to range from 0.7 to 3.1 pg/m^3 in total with around 90 ± 5% associated with particulates. This study also demonstrated that an urban environment was an important factor with respect to atmospheric concentrations. Roosens et al. (2010) reported the exposure of the Flemish population to BFRs from various sources and included HBCDDs in air. They did

not measure concentrations but used a figure of 37 pg/m^3 for outdoor air, 180 pg/m^3 for indoor air (homes) and 175 pg/m^3 for indoor air (offices) based on findings from 12 European studies reported between 2004 and 2008.

There are very little recent data available on HBCDDs in European air. Drage et al. (2016) found that the average ∑HBCDDs air concentration in an urban area in the UK was 100 pg/m^3, but noted that concentrations were not correlated with distance from the city centre. The authors suggested that the high levels and lack of correlation were likely to be due to the sampling protocol which included locations that were in proximity to buildings possibly containing HBCDDs, which was heavily used as a flame retardant in building insulation at the time of construction.

Newton et al. (2015) studied a variety of emerging flame retardants in Swedish indoor and outdoor air, but HBCDDs were only detected in the Central Urban air sample where the concentration for ∑HBCDDs was 0.58 pg/m^3.

UK public microenvironments had the highest indoor air HBCDD concentrations of 900 pg/m^3 (median) compared to 2 pg/m^3 (median) in Swedish houses (Drage et al., 2016).

Levels in dust in Europe that have been reported since the previous Opinion (EFSA CONTAM Panel, 2011a) are summarised in **Appendix A**. For ΣHBCDD, levels reported vary significantly with some as low as < 1 ng/g and others as high as around 150,000 μg/kg. Highest concentrations are generally for α-HBCDD followed by γ-HBCDD and then β-HBCDD, although for some samples, particularly those with the highest concentrations, γ-HBCDD can be the predominant stereoisomer.

Concentrations in the dust from homes ranged between < 3 (de Wit et al., 2012) to over 100,000 μg/kg ΣHBCDD (Tao et al., 2016a; Tay et al., 2017), with the majority of data reported being below 1,000 μg/kg. Levels in offices, day-care facilities and educational establishments were in the same broad range, although they were typically higher than in homes (**Appendix A**). Some of these studies may have focussed on places where high exposure may be anticipated, e.g. aircraft and other vehicles, and in some instances, values over 1,000,000 μg/kg were reported (Allen et al., 2013).

Malliari and Kalantzi (2017) performed a review on children's exposure to BFRs in indoor environments. In general, large geographical variations were observed for all BFRs, including HBCDDs. The authors estimated median worldwide dust levels in samples collected between 2006 and 2014, ranging from 6.15 μg/kg in Egyptian houses to 340 ng/g in Swedish day-care centres, with most median concentrations in the range of 100–300 μg/kg dust.

Occurrence in wildlife

Concentrations of HBCDDs have been reported for terrestrial and marine species of birds, and for marine mammals from both industrialised and remote regions. Concentrations in polar bears from different Arctic regions have also been measured (Muir et al., 2006; ECB, 2008).

HBCDD concentrations in wildlife that have been reported since the previous Opinion (EFSA CONTAM Panel, 2011a) support the findings that were previously stated. Most data generated for wildlife concern marine species (Jin et al., 2015; Houde et al., 2017). There are some data on wild species that are sometimes used for food. Zacs et al. (2018a) measured a range of contaminants in the meat and liver of red deer (*Cervus elaphus*), wild boar (*Sus scrofa*) and moose (*Alces alces*) in a study on Latvian wildlife. The wild boar samples contained the highest levels of HBCDDs, with the mean concentration equal to 0.264 μg/kg ww in muscle tissues. In a different study (Zacs et al., 2018b), emerging BFRs and dechlorane-related compounds were measured in European eels (*Anguilla anguilla*) from Latvian lakes. HBCDDs were found in concentrations up to 6.58 μg/kg lipid in the eels.

There are several studies that have been conducted in fish where the primary focus was as an environmental indicator rather than as a food source. Bustnes et al. (2012) investigated the latitudinal distribution of several POPs including HBCDDs, in livers of two species of marine fish, the pelagic saithe (*Pollachius virens*, n = 40) and the demersal cod (*Gadus morhua*, n = 40), along a south–north gradient (59°–70°N) on the Norwegian coastland. It was found that concentrations of especially heavy halogenated compounds (such as HBCDDs) were higher in fish from the southern-most region than in those taken further north.

Bream was sampled annually from several European freshwater sites over a period of 4 years starting from 2007 by Rüdel et al. (2012). Lowest levels of HBCDDs (11 μg/kg lipid weight, sum of three HBCDD diastereomers) were detected in bream sampled in 2009 from Lake Belau, which is situated in a rural area of Northern Germany. Significant decreases of HBCDDs were detected in bream from the rivers Rhone (France; –85%, to 205 μg/kg lipid weight) and Western Scheldt (the Netherlands; –60%, to 36 μg/kg lipid weight). High concentrations of HBCDDs (9,480–14,500 μg/kg lipid weight) were observed in bream from the River Tees (UK) with no clear changes in concentration

associated with time. A subsequent study (Rüdel et al., 2017) found that for most sites, a decrease in ∑HBCDDs was observed in fish, e.g. in the Rhône and Western Scheldt by about 80 and 60%, respectively, with significantly decreasing trends, $p < 0.01$. At the sampling site in the River Tees, which was impacted by a former HBCDDs point source, concentrations in fish decreased only after a major flood event in 2013.

Munschy et al. (2013) investigated the temporal trend for HBCDDs in mussel (*Mytilus edulis*) samples harvested at the Seine estuary/France between 1981 and 2011. The concentration for α-HBCDD ranged between 0.01 and 0.39 μg/kg wet weight (ww[4]). Although concentrations decreased between 2008 and 2010, over the entire study period (1981–2011) the contamination levels revealed a significant exponential upward trend with a doubling time of 8 years. However, since this time, the impact on restrictions in use of HBCDDs might have changed this trend.

Fliedner et al. (2016) also studied bream using samples from the German environmental specimen bank. Levels were measured and trends were examined in relation to the EU Water Framework Directive on priority substances in freshwater fish. Samples were mostly compliant with the environmental quality standard (EQS) for HBCDDs (167 μg/kg ww; see **Section 1.3.6**) but increasing trends were detected for PBDEs and HBCDDs, particularly at one Saar site located downstream of the industries and conurbation of Saarbrücken and Völklingen. The authors suggested that this finding related to new sources of emissions of BFRs. The same authors analysed georeferenced samples of eelpout (*Zoarces viviparus*) fillet from the German environmental specimen bank for HBCDDs (Fliedner et al., 2018). Two sampling sites were located at German coastal areas of the Central North Sea and one site in the Bodden National Park of Western Pomerania. Catching of the fish was between 2003 and 2017. The levels for HBCDDs ranged between 0.03 and 12.2 μg/kg ww with most samples between 0.11 and 1.5 μg/kg ww. Between 2003 and 2017, significant decreases were observed at two sampling sites ($p = 0.03$ and < 0.01).

Eels were used as a 'bioindicator' species for the determination of the levels of organic contaminants within different Irish water bodies by McHugh et al. (2010). Levels of HBCDDs were between 1.2 and 15 μg/kg (ww), corresponding to 7.4–166 μg/kg (lipid weight), which is in line with concentrations reported elsewhere.

Eljarrat and Barcelo (2018) reviewed the global scientific literature to compare PBDE and HBCDD levels in river fish with the European EQSs. Most results reported were compliant, but two European studies (Rüdel et al., 2012; Malarvannan et al., 2015) and one Chinese study (Tang et al., 2015) reported a range of concentrations up to 751 μg/kg ww, exceeding the EQS for HBCDDs (167 μg/kg ww; **Section 1.3.6**) by a factor of about 5.

Due to the fact that HBCDDs are persistent, lipophilic and bioaccumulate, they can be found in all animal species including humans, in particular in lipid rich compartments, such as adipose tissue, milk and liver (see **Section 3.1.1.4**).

1.3.4. Sampling and methods of analysis

1.3.4.1. Sampling

The primary objective is to obtain a representative and homogeneous laboratory sample with no secondary contamination. Therefore, basic rules for sampling of organic contaminants or pesticides should be followed. Respective requirements are laid down, e.g. in Commission Regulation (EU) No 2017/644[5] laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-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, labelling, interpretation of analytical results and requirements for assessing the compliance of a lot or sublot with the legislation. Commission Recommendation 2014/118/EU on the monitoring of traces of brominated flame retardants in food lays stipulates that Member States should follow the sampling procedures laid down in Annex II of the predecessor version of the Regulation (EU) No 2017/644 in order to ensure that the samples are representative of the sampled lot.

[4] In this Opinion, the terms 'wet weight', 'whole weight' and 'fresh weight' are considered synonymous, unless otherwise stated, are abbreviated as ww.

[5] Commission Regulation (EU) 2017/644 of 5 April 2017 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 589/2014. OJ L 92, 6.4.2017, p. 9–34.

1.3.4.2. Methods of analysis

Detailed information on methods of analysis for HBCDDs in food and parameters that influence the validity of results are described in the previous EFSA Opinion on HBCDDs (EFSA CONTAM Panel, 2011a). In summary, the analytical approaches for the HBCDDs extraction and clean-up generally follow the methodologies for the analysis of persistent lipophilic compounds, such as extraction with organic solvents, pressurised liquid extraction (PLE) and solid phase extraction (SPE). Clean-up is usually performed by different column chromatographic techniques to remove co-extracted interfering matrix constituents and to separate HBCDDs from potentially interfering compounds (Covaci et al., 2007; Xu et al., 2013; Kuc and Grochowalski, 2014). Newer approaches make use of modified QuEChERS (quick, easy cheap, effective rugged and safe) methods (Plassmann et al., 2015; Yuan et al., 2016; Li et al., 2017a).

The analytical determination of HBCDDs is performed either by gas chromatography (GC)/mass spectrometry (MS) or liquid chromatography (LC)/MS-based methods. Both techniques enable the use of isotope-labelled internal standards, which allow for the correction of losses during extraction and clean-up. GC/MS analyses are, however, hampered by the fact that a separation of the stereoisomers is not possible by this approach. Thus, the result is reported as Total HBCDDs.

The separate specific analysis of the HBCDD stereoisomers as well as their enantiomers is performed by LC/MS-based methods. The analyses are often integrated into the procedures for the determination of a number of other brominated flame retardants, such as TBBPA and bromophenols (Zhou et al., 2010; Butt et al., 2016; Bichon et al., 2018). Baek et al. (2017) reported that δ- and ε-HBCDD can co-elute with the predominant stereoisomers α-, β- and γ-HBCDD on columns with C18 stationary phases which are chosen in most studies for HBCDD analysis. In order to avoid false-positive results, they recommend a phenyl-hexyl ultra-high-performance LC (UPLC) column on which 10 HBCDD diastereomers can be resolved. The advances in the enantioselective analysis of HBCDDs and other chiral flame retardants with special emphasis on current status, limitations and future perspectives were reviewed by Badea et al. (2016). The CONTAM Panel noted that this potential co-elution is of minor importance for the analysis of food, as δ- and ε-HBCDD may be present at only low concentrations in the technical product.

LC-MS detection is mostly performed on triple-quadrupole instruments (MS/MS) using electrospray ionisation (ESI) negative mode. Covaci et al. (2007) and Guerra et al. (2011) give extensive overviews on respective methods used for the determination of HBCDDs in various matrices.

In the past few years, new analytical instrumentation for the analysis of HBCDDs and other BFRs was introduced. These include *inter alia* the coupling of supercritical fluid chromatography to positive ion atmospheric pressure ionisation mass spectrometry (Riddell et al., 2017), the application of GC-atmospheric pressure chemical ionisation (APCI)-MS/MS (Sales et al., 2016), LC-atmospheric pressure photoionisation (APPI)-Orbitrap mass spectrometry (Zacs and Bartkevics, 2015) and ultra-high-performance LC-time-of-flight (TOF) high resolution MS (Zacs et al., 2014).

1.3.4.3. Analytical quality assurance

The analysis of HBCDDs in food is complex and involves several critical steps. As mentioned earlier, exposure to high temperature and to UV irradiation should be avoided as this may lead to breakdown of the parent compound and/or transformation from γ- to α-HBCDD. It is therefore recommended to perform all analytical steps in amber glassware or in glassware covered with aluminium foil.

A prerequisite for laboratories to demonstrate that their applied methods of analysis are fit for purpose is the successful participation in proficiency tests or interlaboratory studies. Information on interlaboratory studies performed in biota and food samples before 2010 are summarised in the EFSA Opinion on HBCDDs of 2011 (EFSA CONTAM Panel, 2011a). The European Reference Laboratory (EURL) for Halogenated Persistent Organic Pollutants (POPs) in Feed and Food as well as the Norwegian Institute of Public Health (NIPH) regularly offer proficiency tests for analysing various persistent organic pollutants, including HBCDDs in feed and food.

The recent proficiency test which included the analysis of HBCDDs was organised by the EURL for National Reference Laboratories (NRLs) and official laboratories, and concerned cod liver and fish liver oil in 2014, bovine liver in 2017, soy bean meal and beef in 2018 and grass in 2019. In the 2014 EURL proficiency test on cod liver and fish oil, assigned values of each 1.1 ng/g ww for α-HBCDD were estimated. The share of results with a z-score[6] $\leq$ 2 was 80% for cod liver and 90% for fish liver oil. In

[6] A z-score is a numerical measurement used in statistics of a value's relationship to the mean (average) of a group of values, measured in terms of standard deviations from the mean.

the EURL grass PT in 2019, the median concentrations for α-, β- and γ-HBCDD each were around 0.1 μg/kg (88% dry matter). About 90% of the submitted results were within ± 40% of the median value (Schächtele, 2019).

The interlaboratory comparison studies offered and organised by the NIPH are open to all laboratories whether official or private. Laboratories were asked in each case to provide results on HBCDDs in addition to data on dioxins and PCBs. Besides a standard solution submitted by the organisers in each round, the matrices involved were reindeer meat, halibut filet and cod liver oil in 2012, poultry meat, crab meat and eggs in 2013, pork, herring and cow's milk in 2014, beef, salmon and cheese in 2015, sheep liver, salmon and fish oil in 2016, sheep meat, cod liver and herring in 2017, and reindeer meat, salmon and fish oil in 2018. Generally, 5–15 laboratories reported one or more of the three isomers in the various food samples. The organisers state that due to the low number of laboratories, no consensus values could be derived and thus the results must be regarded as indicative values.

In the 2017 NIPH interlaboratory comparison study, the median concentrations (all values) for the sum of α-, β- and γ-HBCDD were 10 pg/g ww for the sheep meat sample, 2,950 pg/g ww for cod liver and 376 pg/g ww for the herring sample. The evaluation of the results indicate that the relative standard deviations substantially increase with decreasing concentrations, being 14%, 33% and 227% for the sum of α-, β- and γ-HBCDD in the cod liver, herring and sheep meat samples.

A similar outcome was reported for the samples analysed within the 2018 NIPH interlaboratory comparison study. The median concentrations (all values) for the sum of α-, β- and γ-HBCDD were 11 pg/g ww for the reindeer meat sample, 378 pg/g ww for the salmon and 1,519 pg/g ww for the fish oil sample. The respective relative standard deviations were 19% for salmon, 45% for fish oil and 134% for the reindeer sample.

All results of the various interlaboratory comparison studies are published on the home page of the NIPH.[7]

1.3.5. Previous risk assessments

In 2011, the EFSA CONTAM Panel published its risk assessment on HBCDDs in food (EFSA CONTAM Panel, 2011a). The Panel identified as the critical endpoint neurodevelopmental effects on behaviour and derived a $BMDL_{10}$ of 0.93 mg/kg bw, based on the study by Eriksson et al. (2006) in mice, using a single oral administration on postnatal day 10 (PND10). Because the elimination kinetics for HBCDDs between experimental animals and humans differ, the CONTAM Panel converted this $BMDL_{10}$ into an estimated chronic human dietary intake associated with the body burden at the $BMDL_{10}$ as the basis for the risk assessment. For this, the Panel first estimated the body burden at the $BMDL_{10}$ (0.79 mg/kg bw, based on 85% oral absorption in mice). Second, the chronic human dietary intake associated with the body burden at the $BMDL_{10}$ was calculated, assuming the human absorption of HBCDDs to be 100%, in the absence of robust information on the actual absorption, and using the 'worst-case' longest human half-life identified of 219 days. This resulted in an estimated human dietary intake associated with the body burden at the $BMDL_{10}$ of 0.003 mg/kg bw per day. Due to the limitations and uncertainties in the database, the CONTAM Panel did not find it appropriate to establish a health-based guidance value (HBGV), and instead used a margin of exposure (MOE) approach for the risk characterisation. The Panel considered that an MOE larger than 8 would imply no health concern, as it would cover interspecies differences on toxicodynamics for the effect observed (uncertainty factor of 2.5) and intraspecies differences for toxicokinetics due to the uncertainty in the elimination half-life in humans (factor of 3.2). The Panel considered that since the MOE approach was based on a body burden comparison between animals and humans, the potential kinetic differences between animals and humans had been accounted for. And that 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 differences in susceptibility had been covered. The chronic human dietary intake of 0.003 mg/kg bw per day was compared with the estimate of the dietary exposure for different age groups based on HBCDD levels in food collected in seven European countries covering the period from 2000 to 2010. The CONTAM Panel concluded that the dietary exposure as estimated at the time of the assessment did not raise a health concern based on the MOEs obtained.

[7] https://www.fhi.no/en/sys/search-result/?term=interlaboratory%20comparison

Since then, several bodies have performed risk assessments for HBCDDs. The details of these assessments are reported in **Table 2**.

Some of these assessments used the chronic human dietary intake associated with the body burden at the $BMDL_{10}$ for neurodevelopmental effects calculated by the CONTAM Panel in its 2011 assessment, without reviewing the new studies since its publication (Petersen et al., 2013; Rivière et al., 2014). Others did review the new toxicological studies available since the EFSA Opinion but concluded that the new studies did not offer an alternative approach to the Reference Point identified by EFSA and its corresponding chronic human dietary intake (COT, 2015; ANSES, 2017).

Other bodies identified NOAELs/LOAELs from different studies for their risk assessments. Environment Canada/Health Canada (2011) identified a NOAEL of 10 mg/kg bw per day from a two-generation reproductive toxicity study (Ema et al., 2008) to assess the risk for the adult population, while for infants and children identified a lowest-observed-adverse-effect level (LOAEL) of 0.9 mg/kg bw per day based on the study by Eriksson et al. (2006). The National Industrial Chemicals Notification and Assessment Scheme (NICNAS, 2012) identified a NOAEL of 10.2 mg/kg bw per day based on the Ema et al. (2008) study, that was used for both adults and children risk characterisation. None of these bodies established a HBGV but calculated the MOE, i.e. the margin between the $BMDL_{10}$ or NOAEL/LOAELs identified and the estimated exposure in the corresponding country. All these risk assessments concluded that the calculated MOEs indicate that the respective exposure levels are of no health concern.

The Japanese National Institute of Technology and Evaluation (NITE, 2019) identified an NOAEL of 10 mg/kg bw per day based on the two-generation reproduction toxicity study (Ema et al., 2008), and derived hazard assessment values[8] for general toxicity (0.050 mg/kg bw per day, based on increased absolute and relative liver weight) and for reproductive and developmental toxicity (0.10 mg/kg bw per day, based on a reduction of the number of primordial follicles in the ovary of F1 females).

In 2014, the US-EPA's IRIS (Integrated Risk Information System) released a draft preliminary assessment on HBCDDs prior to the development of the draft assessment (US-EPA, 2014). It included, among others, the draft literature searches and tables summarising the critical available information for HBCDDs, to obtain input from the public prior to developing the draft IRIS assessment. However, in 2019, US-EPA has prioritised its IRIS assessments, and the assessment for HBCDDs among others, have been discontinued.[9]

[8] According to the authors (NITE, 2019), the hazard assessment values were derived by dividing the NOAEL by uncertainty factors: the hazard assessment value of 0.050 mg/kg bw per day for general toxicity was derived by dividing the NOAEL by an uncertainty factor of 200 (10 for animal-to-human extrapolation, 10 for human variability and 2 in consideration of the use of NOAEL as well as the test period). The hazard assessment value of 0.10 mg/kg bw per day for reproductive and developmental toxicity (reduction of the number of primordial follicles) was derived by dividing the NOAEL by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability).

[9] IRIS Programme Outlook (April 2019). Available online: https://www.epa.gov/iris/iris-program-outlook

Table 2: (Risk) Assessments on HBCDDs since the publication of the EFSA CONTAM Panel (2011a) Opinion, including details about the latter for comparison

Reference	Country	Compounds	NOAEL/LOAEL/BMD	HBGV/MOE	Dietary exposure (ng/kg bw per day)	Conclusion
EFSA CONTAM Panel (2011a)	Europe	Sum of α-, β- and γ-HBCDD	$BMDL_{10}$ = 0.93 mg/kg bw (based on neurodevelopmental effects, single dose at PND10, Eriksson et al., 2006) Associated chronic intake = 0.003 mg/kg bw per day (related to the body burden at the $BMDL_{10}$)	MOE An MOE larger than 8 was considered to imply no health concern, based on UFs of 2.5 for interspecies differences on toxicodynamics and 3.2 for intraspecies differences for toxicokinetics	Mean exposure[(a)]: Children: 0.15–1.85 Adults: 0.09–0.99 Very Elderly: 0.06–0.54 P95 exposure[(a)]: Children: 0.80–4.46 Adults:0.39–2.07 Very Elderly: 0.27–1.26 Specific population groups: High consumers of fish: 2.76[(b)] Consumers of fish liver (once a week): 1.94[(b)]	'*The CONTAM Panel concluded that current dietary exposure to HBCDDs in the European Union does not raise a health concern*'
Rivière et al. (2014)	France	Sum of α-, β- and γ-HBCDD	Did not review new literature on toxicity. Used EFSA CONTAM Panel (2011a) chronic dietary intake associated with the body burden at the $BMDL_{10}$ (see above)	MOE	Total Diet Study Adults: 0.211 Children: 0.320	'*The exposure levels are deemed to be of no public health concern (i.e. MOEs higher than 1000 for children and adults for the 95th percentiles)*'
Petersen et al. (2013)	Denmark	Sum of α-, β- and γ-HBCDD	Did not review new literature on toxicity. Used EFSA CONTAM Panel (2011a) chronic dietary intake associated with the body burden at the $BMDL_{10}$ (see above)	MOE	Fish Adults mean exposure = 0.19 95th percentile = 0.75 Children (4–14 years old): Mean exposure = 0.23 95th percentile = 1.28	'*The estimated MOEs are of no food safety concern*'

Reference	Country	Compounds	NOAEL/LOAEL/BMD	HBGV/MOE	Dietary exposure (ng/kg bw per day)	Conclusion
COT (2015)	UK	Sum of α-, β- and γ-HBCDD	Reviewed new toxicity data since EFSA CONTAM Panel (2011a): '*Overall, the new data did not demonstrate a mode of action for HBCDDs or provide an improved basis for extrapolation of experimental animal data to the human situation. Thus, they did not offer an alternative approach to the reference point identified by EFSA from the study by Eriksson* et al. *(2006), which remained the best available starting point for risk assessment*' Reference Point = 3 μg/kg bw per day $BMDL_{10}$ body burden = 0.79 mg/kg bw (based on neurodevelopmental effects, Eriksson et al., 2006)	MOE	Breast milk, food for infants Breastfed infants (median): Average consumers (800 mL/day) 0–4 months: 0.018 > 4–6 months: 0.014 High consumers: 0–4 months: 0.027 > 4–6 months: 0.021 Infants: 4–6 months: 1.39 6–9 months: 1.62 9–12 months: 1.74	'*Overall the analysis indicated that estimated exposures via breast milk and food are not a cause for concern, but that high levels found in some samples of domestic dust are*'
ANSES (2017)	France	Sum of α-, β- and γ-HBCDD	Reviewed new toxicity data since EFSA CONTAM Panel (2011a). Used EFSA CONTAM Panel (2011a) chronic dietary intake associated with the body burden at the $BMDL_{10}$ (see above).	MOE	Mean UB: Infants (1–4 months): 8.27 Infants (1–3 years): 0.505 Children (3–17 years): 0.320 Adults: 0.211 P90 UB: Infants (1–4 months): 43.2 Infants (1–3 years): 0.880 P95 UB: Adults: 0.448 Children (3–17 years): 0.734	'*Based on current knowledge and the available data, exposure via food of the children population to the sum of HBCDD is considered tolerable*'

Reference	Country	Compounds	NOAEL/LOAEL/BMD	HBGV/MOE	Dietary exposure (ng/kg bw per day)	Conclusion
Environment Canada/Health Canada (2011)	Canada	HBCDDs	To assess the risk for the adult population: NOAEL = 10 mg/kg bw per day (based on a two-generation reproductive toxicity study, Ema et al., 2008). To assess the risk for infants and children: LOAEL = 0.9 mg/kg bw per day (based on altered behaviour in mice, Eriksson et al., 2006).	MOE	UB exposure = 0.042 μg/kg bw per day (including food, ambient air and dust) Breastfed infants = 0.089 μg/kg bw per day	'*Comparison with the NOAEL of 10 mg/kg bw per day resulted in a margin of exposure of 240,000. For breastfed infants, the margin was 10,000. These margins were considered by Environment Canada to be protective for breastfed children and adequate to address uncertainties in the exposure and health effects database*.'
NICNAS (2012)	Australia	HBCDDs	NOAEL = 10.2 mg/kg bw per day (based on a two-generation reproductive toxicity study, Ema et al., 2008)	MOE	Food consumption Typical exposure: Toddlers = 24 Children = 6 Adults =5.9 Reasonable worst-case: Toddlers = 50 Children = 12 Adults =12	'*low risk from exposure through food*'
NITE (2019)	Japan	HBCDDs	NOAEL = 10 mg/kg bw per day (based on a two-generation reproduction toxicity study, Ema et al., 2008)	Hazard assessment value[(f)] for general toxicity (increase absolute and relative liver weight) = 0.050 mg/kg bw per day[(c)] Hazard assessment value for reproductive and developmental toxicity (reduction of the number of primordial follicles in the ovary of F1 females) = 0.10 mg/kg bw per day[(d)]	_[(e)]	_

$BMDL_{10}$: benchmark dose lower confidence limit for a benchmark response of 10%; HBGV: health-based guidance value; LOAEL: lowest observed adverse effect level; MOE: margin of exposure; TDS: total diet study; NOAEL: no observed adverse effect level; PND: postnatal day.

(a): Mean exposure across dietary surveys in European countries.
(b): Maximum UB across European surveys.
(c): Was derived by dividing the NOAEL by an UF of 200 (10 for animal-to-human extrapolation, 10 for human variability and 2 in consideration of the use of NOAEL as well as the test period (NITE, 2019).
(d): Was derived by dividing the NOAEL by an uncertainty factor of 100 (10 for animal-to-human extrapolation and 10 for human variability) (NITE, 2019).
(e): No dietary exposure was estimated.
(f): According to the authors (NITE, 2019), the hazard assessment values were derived by dividing the NOAEL by an uncertainty factor of 200 (10 for animal-to-human extrapolation, 10 for human variability and 2 in consideration of the use of NOAEL as well as the test period).

1.3.6. Legislation

In this Opinion, where reference is made to European legislation the reference should be understood as relating to the most recent amendment at time of publication of this Opinion, unless otherwise stated.

In order to protect public health, Article 2 of Council Regulation (EEC) No 315/93[10] of 8 February 1993 laying down Community procedures for contaminants in food stipulates that, where necessary, maximum tolerances for specific contaminants shall be established. A number of maximum levels (MLs) are currently laid down in Commission Regulation (EC) No 1881/2006.[11] HBCDDs are not regulated so far under this Regulation or under any other specific European Union (EU) regulation for food.

Council Directive 2002/32/EC[12] regulates undesirable substances in animal feed. HBCDDs are, so far, not regulated under this Directive or any other specific EU regulation for feed.

In 2013 and following the recommendation from the Persistent Organic Pollutants Review Committee (POPRC), HBCDDs[13] were listed in Annex A (Elimination) of the Stockholm Convention, with specific exemptions for use or production.[14] For chemicals listed in Annex A, countries must take measures to eliminate the production and use of it.

HBCDDs are listed in Annex I, Part A of Regulation (EU) 2019/1021 of the European Parliament and of the Council on persistent organic pollutants (POPs).[15] The objective of this Regulation is to protect human health and the environment by prohibiting, phasing out as soon as possible or restricting the manufacturing, placing on the market and use of POPs.

HBCDDs and all major diastereoisomers identified (α-, β- and γ-) are included in Annex XIV of Regulation (EC) No 1907/2006 (REACH). According to Art. 57 (d), they are identified as PBT (persistent, bioaccumulative and toxic). The latest application date and sunset date were set as 21 February 2014 and 21 August 2015, respectively, and it is in the candidate list of substances of very high concern (SVHC) for authorisation.[16]

According to Annex VI of Regulation (EC) No 1272/2008 (Classification, Labelling and Packaging (CLP) Regulation), HBCDDs and 1,2,5,6,9,10-HBCDD are classified (harmonised classification) Repr. 2 H 361 (suspected of damaging fertility or the unborn child) and Lact. H 362 (may cause harm to breastfed children). For the stereoisomers, there are notified classifications (see **Table 3**).[17]

Table 3: Harmonised classification of HBCDDs (source: ECHA)

Compound	EC/List no	CAS no	Hazard Classification
HBCDD	247-148-4	25637-99-4	The harmonised classifications are Repr. 2 H 361 (suspected of damaging fertility or the unborn child) and Lact. H 362 (may cause harm to breastfed children).
α-HBCDD	603-801-9	134237-50-6	The notified classifications are Repr. 2 H 361 (suspected of damaging fertility or the unborn child) and Lact. H 362 (may cause harm to breastfed children).
β-HBCDD	603-802-4	134237-51-7	The notified classifications are Skin Irrit. 2 H 315 (causes skin irritation), Eye Irrit. 2 H 319 (causes serious eye irritation), and STOT SE 3 H 335 (may cause respiratory irritation).
ϒ-HBCDD	603-804-5	134237-52-8	The notified classifications are Repr. 2 H 361 (suspected of damaging fertility or the unborn child) and Lact. H 362 (may cause harm to breastfed children).

[10] 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. 1–3.
[11] 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.
[12] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in food. OJ L 140, 30.2.2002, p. 10–22.
[13] According to the Stockholm Convention, 'Hexabromocyclododecane' means hexabromocyclododecane (CAS No: 25637-99-4), 1,2,5,6,9,10-hexabromocyclododecane (CAS No: 3194- 55-6) and its main diastereoisomers: alpha- hexabromocyclododecane (CAS No: 134237-50-6); beta-hexabromocyclododecane (CAS No: 134237-51- 7); and gamma-hexabromocyclododecane (CAS No: 134237-52-8).
[14] http://chm.pops.int/Implementation/Alternatives/AlternativestoPOPs/ChemicalslistedinAnnexA/tabid/5837/Default.aspx
[15] Regulation (EU) 2019/1021 of 20 June 2019 on persistent organic pollutants. OJ L 169, 25.6.2019, p. 45–77.
[16] https://echa.europa.eu/documents/10162/471aceac-4e5e-4c53-a4b2-23159a290893
[17] https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/104361

Compound	EC/List no	CAS no	Hazard Classification
1,2,5,6,9, 10-HBCDD	221-695-9	3194-55-6	The harmonised classifications are Repr. 2 H 361 (suspected of damaging fertility or the unborn child) and Lact. H 362 (may cause harm to breastfed children).

HBCDDs are identified as priority hazardous substances under Directive 2013/39/EU of the European Parliament and of the Council amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.[18] Environmental quality standards (EQS, e.g. annual average and maximum allowable concentrations) are laid down in surface water and biota for HBCDDs.[19] These refer to 1,3,5,7,9,11-hexabromocyclododecane[20] (CAS 25637-99-4), 1,2,5,6,9,10-hexabromocyclododecane (CAS 3194-55-6), α-hexabromocyclododecane (CAS 134237-50-6), β-hexabromocyclododecane (CAS 134237-51-7) and γ-hexabromocyclododecane (CAS 134237-52-8). In the Annex of this Directive maximum allowable concentrations (MAC-EQS) for inland surface waters (rivers, lakes and related artificial or heavily modified water bodies), and other surface waters of 0.5 μg/L and 0.05 μg/L, respectively, are set. For fish, an EQS of 167 ng/g is established.

In Commission Recommendation 2014/118/EU[21], the EU Commission recommended that Member States should perform monitoring on the presence of brominated flame retardants in food following the recommendations of the previous EFSA risk assessment on HBCDDs and other BFRs (EFSA CONTAM Panel, 2011a,b, 2012a,b,c). Besides various other brominated flame retardants, the Recommendation also includes the α-, β- and γ-stereoisomers of 1,2,5,6,9,10-HBCDD. The aim of the monitoring is to include a wide variety of individual foodstuffs reflecting consumption habits in order to give an accurate estimation of exposure. Regarding HBCDDs, it is recommended to analyse fish and other seafood, meat and meat products, milk and dairy products, eggs and egg products, as well as infant and follow-up formula. The analytical methods should allow the stereoisomeric determination of the above-mentioned three HBCDDs with limits of quantification of 0.01 ng/g ww or lower.

2. Data and methodologies

The current updates of the EFSA risk assessments on BFRs in food, including the current one on HBCDDs, were developed applying a structured methodological approach, which implied developing a priori the protocol or strategy of the full risk assessments and performing each step of the risk assessment in line with the strategy and documenting the process. The protocol in **Annex A** to this Opinion contains the method that was proposed for all the steps of the risk assessment process, including any subsequent refinements/changes made.

The CONTAM Panel used its previous risk assessment on HBCDDs in food (EFSA CONTAM Panel, 2011a) as a starting point for drafting the current Opinion.

2.1. Supporting information for the assessment

Information on physico-chemical properties, production and industrial use, environmental fate and levels, analytical methods, previous assessment and legislation was gathered from the previous EFSA Opinion on HBCDDs (EFSA CONTAM Panel, 2011a), assessments by international bodies (by checking their original websites of the relevant organisations) and from current EU legislation. Literature searches were conducted to identify new information in reviews and peer-reviewed publications. Details about the literature searches are given in **Appendix B**. The information was summarised in a narrative way based on expert knowledge and judgement.

[18] Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy Text with EEA relevance. OJ L 226, 24.8.2013, p. 1–17.

[19] Referring to 1,3,5,7,9,11-Hexabromocyclododecane (CAS 25637-99-4), 1,2,5,6,9,10- Hexabromocyclododecane (CAS 3194-55-6), α-Hexabromocyclododecane (CAS 134237-50-6), β-Hexabromocyclododecane (CAS 134237-51-7) and γ- Hexabromocyclododecane (CAS 134237-52-8).

[20] Considering the manufacturing process for HBCDDs, the formation of a structure described by the name 1,3,5,7,9,11-hexabromocyclododecane is unlikely. No REACH registration has been requested for a product with this substitution pattern.

[21] Commission Recommendation 2014/118/EU of 3 March 2014 on the monitoring of traces of brominated flame retardants in food. OJ L 65, 5.3.2014, p. 39–40.

2.2. Hazard identification and characterisation

Information relevant for the sections under hazard identification and characterisation was identified by a literature search to gather review studies and primary research studies. Details about the literature search are given in **Appendix B**. The information retrieved was screened and evaluated by relevant domain experts from the EFSA CONTAM Working Group on BFRs in food and used for the present assessment. The selection of the scientific papers for inclusion or exclusion was based on consideration of the extent to which the study was relevant to the assessment or on general study quality considerations (e.g. sufficient details on the methodology, performance and outcome of the study, on dosing, substance studied and route of administration and on statistical description of the results), irrespective of the results. Limitations in the information used are documented in this Scientific Opinion.

Benchmark dose (BMD) analysis was carried out according to the latest EFSA guidance (EFSA Scientific Committee, 2017). To perform the BMD modelling, EFSA used the web-app 'EFSA-Proast platform' (https://efsa.openanalytics.eu/) that is based on the PROAST software package developed by RIVM. Further information about PROAST can be found at: https://www.rivm.nl/en/proast.

The draft scientific Opinion underwent a public consultation from 14 October 2020 to 25 November 2020. The comments received and how they were taken into account when finalising the scientific Opinion are available in Annex E.[22]

2.3. Occurrence data submitted to EFSA

The general steps followed for the acquisition of the food occurrence and consumption data for the exposure assessment of BFRs in food are documented in **Annex A**. Specific details on the occurrence data on HBCDDs in food submitted to EFSA are described below.

2.3.1. Data collection and validation

Following an European Commission mandate to EFSA, a call for annual collection of chemical contaminant occurrence data in food and feed, including HBCDDs, was issued by the former EFSA Dietary and Chemical Monitoring Unit (now Evidence Management Unit[23]) in December 2010[24] with a closing date of 1 October of each year. European national authorities and similar bodies, research institutions, academia, food business operators and other stakeholders were invited to submit analytical data on HBCDDs in food.

The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description (SSD) for Food and Feed (EFSA, 2010a). Occurrence data were managed following the EFSA standard operational procedures (SOPs) on 'Data collection and validation' and on 'Data analysis of food consumption and occurrence data'.

Data on HBCDDs in food available in the EFSA database from 2010 until the end of December 2018 were used for the present assessment.

The raw occurrence dataset on HBCDDs as extracted from the EFSA data warehouse is available at the EFSA Knowledge Junction community on Zenodo.[25]

2.3.2. Data analysis

Following EFSA's SOP on 'Data analysis of food consumption and occurrence data' to guarantee an appropriate quality of the data used in the exposure assessment, the initial data set was carefully evaluated by applying several data cleaning and validation steps. Special attention was paid to the identification of duplicates and to the accuracy of different parameters, such as 'Sampling strategy', 'Analytical methods', 'Result express' (expression of results, e.g. fat weight), 'Reporting unit', 'Limit of detection/quantification' and the codification of analytical results under FoodEx classification (EFSA, 2011a). The outcome of the data analysis is presented in **Section 3.2.1**.

The left-censored data (analytical data below the LOD or limit of quantification (LOQ)) were treated by the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of

[22] The outcome of the public consultation was endorsed by the CONTAM Panel in the form of a Technical Report at its 112th Plenary meeting held 26 January 2021. Due to the implementation of the new OpenEFSA portal there has been a change and the outcome of the public consultation is now reported as an Annex to the Opinion, and the Question number initially assigned to the Technical Report is no longer of use.

[23] From 1 January 2014 onwards, Evidence Management Unit (DATA).

[24] http://www.efsa.europa.eu/en/consultations/call/190410

[25] https://doi.org/10.5281/zenodo.4475651

Chemicals in Food' (WHO/IPCS, 2009). The same method is described in the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010b) as an option for the treatment of left-censored data. The guidance suggests that the lower bound (LB) and upper bound (UB) approach should be used for chemicals likely to be present in the food. At the LB, results below the LOQ or LOD were replaced by zero; at the UB, the results below the LOD were replaced by the numerical values of the LOD and those below the LOQ were replaced by the value reported as LOQ.

2.4. Food consumption data

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) provides a compilation of existing national information on food consumption at individual level and was first built in 2010 (EFSA, 2011a; Huybrechts et al., 2011; Merten et al., 2011). Details on how the Comprehensive Database is used are published in the Guidance of EFSA (EFSA, 2011a). The latest version of the Comprehensive Database, updated in 2020, contains results from a total of 69 different dietary surveys carried out in 25 different Member States covering 134,929 individuals.

Within the dietary studies, subjects are classified in different age classes as follows:

Infants:	< 12 months old
Toddlers:	≥ 12 months to < 36 months old
Other children:	≥ 36 months to < 10 years old
Adolescents:	≥ 10 years to < 18 years old
Adults:	≥ 18 years to < 65 years old
Elderly:	≥ 65 years to < 75 years old
Very elderly:	≥ 75 years old

Seven additional surveys provided information on specific population groups: 'Pregnant women' (Austria: ≥ 19 years to ≤ 48 years old, Cyprus: ≥ 17 years to ≤ 43 years old; Latvia: ≥ 15 years to ≤ 45 years old, Spain: ≥ 21 years to ≤ 46 years old, Portugal: 17 years old to 46 years old) and 'Lactating women' (Greece: ≥ 28 years to ≤ 39 years old, Estonia: 18 years old to 45 years old).

For chronic exposure assessment, food consumption data were available from different dietary surveys carried out in 23 different European countries.[26] When for one particular country and age class two different dietary surveys were available, only the most recent one was used. This resulted in a total of 44 dietary surveys selected to estimate chronic dietary exposure.

In **Annex B** (Table B.4), these dietary surveys and the number of subjects available for the chronic exposure assessment are described.

The food consumption data gathered by EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. Consumption data were collected using single or repeated 24- or 48-h dietary recalls or dietary records covering from 3 to 7 days per subject. Because of the differences in the methods used for data collection, direct country-to-country comparisons can be misleading.

2.5. Food classification

Consumption and occurrence data were classified according to the FoodEx classification system (EFSA, 2011b). FoodEx is a food classification system developed by EFSA in 2009 with the objective of simplifying the linkage between occurrence and food consumption data when assessing the exposure to hazardous substances. The system consists of a large number of individual food items aggregated into food groups and broader food categories in a hierarchical parent-child relationship. It contains 20 main food categories (first level), which are further divided into subgroups having 140 items at the second level, 1,261 items at the third level and reaching about 1,800 endpoints (food names or generic food names) at the fourth level.

2.6. Exposure assessment

The CONTAM Panel considered that only chronic dietary exposure to HBCDDs had to be assessed. As suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011c), dietary surveys with only one day per subject were not considered for chronic exposure as they are not

[26] Dietary data included in the assessment were submitted to EFSA when the UK was a member of the EU.

adequate to assess repeated exposure. Similarly, subjects who participated only one day in the dietary studies, when the protocol prescribed more reporting days per individual, were also excluded for the chronic exposure assessment. Not all countries provided consumption information for all age groups, and in some cases, the same country provided more than one consumption survey.

For calculating the chronic dietary exposure to HBCDDs, food consumption and body weight data at the individual level were accessed in the Comprehensive Database. Occurrence data and consumption data were linked at the relevant FoodEx level.

The mean and the high (95th percentile) chronic dietary exposures were calculated by combining mean occurrence values for each food collected in different countries (pooled European occurrence data) with the average daily consumption of each food at individual level in each dietary survey and age class. Consequently, individual average exposures per day and body weight were obtained for all individuals. Based on the distributions of individual exposures, the mean and 95th percentile exposures were calculated per survey and per age class. Dietary exposure was assessed using overall European LB and UB mean occurrence of HBCDDs. All analyses were run using the SAS Statistical Software (SAS enterprise guide 7.15).

2.7. Risk characterisation

The general principles of the risk characterisation for chemicals in food as described by WHO/IPCS (2009) will be applied as well as the different EFSA guidance documents relevant to this step of the risk assessment (see **Annex A**).

3. Assessment

3.1. Hazard identification and characterisation

3.1.1. Toxicokinetics

3.1.1.1. Toxicokinetic studies in experimental animals

Information on the toxicokinetics of HBCDDs is limited. Since the previous EFSA assessment (EFSA CONTAM Panel, 2011a), new information has become available regarding the toxicokinetics. The text below gives a description of previous knowledge complemented with new data. In general, human data about the toxicokinetics of HBCDDs are less abundant than in experimental animal species.

3.1.1.1.1. Absorption

Mice

Szabo et al. (2010, 2011a) and Sanders et al. (2013) studied the toxicokinetics of γ-[^{14}C]-HBCDD (Szabo et al., 2010), α-[^{14}C]-HBCDD (Szabo et al., 2011a) and β-[^{14}C]-HBCDD (Sanders et al., 2013) in female C57BL/6 mice. Mice (n = 4–8) were given a single dose of HBCDD (3, 10, 30 or 100 mg/kg bw) by oral gavage, and urine and faeces were collected daily for 4 days. Another group of female C57BL/6 mice (n = 6–8) received an i.v. single dose (3 mg/kg bw).

By comparing kinetics and disposition between i.v. and oral routes of exposure, the authors calculated the percentage of the three stereoisomers that was absorbed into the systemic circulation 4 days after treatment. The absorption of the three stereoisomers estimated by the authors after oral gavage was around 90%, > 85% and 83% for α-[^{14}C]-HBCDD, β-[^{14}C]-HBCDD and γ-[^{14}C]-HBCDD, respectively.

Rats

Hakk (2016) administered α-, β- and γ-[^{14}C]-HBCDD individually at an oral dose of 3 mg/kg bw to Sprague-Dawley rats (n = 3 rats per isomer). Urine and faeces were collected daily over 96 h and analysed, including animal tissue for radioactivity quantification. The authors estimated an oral absorption of 73–83% for the three isomers (79.7%, 83.2% and 72.9% of the administered dose, respectively, for α-, β- and γ-[^{14}C]-HBCDD).

3.1.1.1.2. Distribution

Mice

The distribution differs between the isomers (Szabo et al., 2010, 2011a; Sanders et al., 2013). Szabo et al. (2011a) studied the distribution of α-[^{14}C]-HBCDD in female C57BL/6 mice following a

single (3, 10, 30 or 100 mg/kg) and 10-day repeated (3 mg/kg) exposure, and showed that α-HBCDD was distributed in the following tissues (for the 10-day repeated exposure at 3 mg/kg): adipose tissue > liver > skin > blood > muscle > lung > brain > spleen > kidney > thymus (other tissues were not reported/analysed by the authors).

Szabo et al. (2010) performed the same study design with γ-[^{14}C]-HBCDD. After a single oral exposure to γ-[^{14}C]-HBCDD (3, 10, 30 or 100 mg/kg) and 10-day repeated exposure (3 mg/kg), the distribution of radioactivity was found in the following tissues (for the 10-day repeated exposure at 3 mg/kg): liver > skin > blood > muscle > lung > kidney > brain > adrenals = thymus > adipose tissue > spleen > bladder (other tissues were not reported/analysed by the authors).

Based on the same protocol as Szabo et al. (2010, 2011a), Sanders et al. (2013) showed that after single oral exposure to β-[^{14}C]-HBCDD (3, 10 or 30 mg/kg), β-HBCDD was distributed in the following tissues (24 h after a single dose of 3 mg/kg β-HBCDD): adipose > muscle > liver > skin > blood > kidney > lung > brain (other tissues were not reported/analysed by the authors).

Neonatal mice

Szabo et al. (2011b) performed a toxicokinetic study on neonatal mice (PND10) with a single oral dose of 3 mg/kg bw of α- and γ-[^{14}C]-HBCDD. They compared their results with a previous experiment on adult mice (PND60) (Szabo et al., 2010, 2011a). They observed a parallel distribution of both isomers (adipose, blood, brain, kidney, muscle and skin) in neonate and adult mice, but α-[^{14}C]-HBCDD and γ-[^{14}C]-HBCDD levels were higher in pups than in adults.

Rats

Hakk (2016) showed that α-, β- and γ-[^{14}C]-HBCDD, when administered individually as a single oral dose of 3 mg/kg bw to Sprague-Dawley rats, were distributed preferentially to adipose tissue, adrenals, skin and gastrointestinal tract 96 h after administration in the following tissues: For α-[^{14}C]-HBCDD: adipose > gastrointestinal tract and content > skin > liver > muscle > kidney = testes > adrenals > lung > thymus = spleen > heart = brain = plasma = heart. For β-[^{14}C]-HBCDD: gastrointestinal tract and content > kidney > liver > adipose > testes > lung > muscle > adrenals > plasma > heart > spleen > brain = thymus. For γ-[^{14}C]-HBCDD: gastrointestinal tract and content > adipose > skin > liver > muscle > kidney > adrenals > plasma = testes > spleen > thymus = heart > lung > muscle > adrenals > plasma > brain = thymus > brain.

In a 90-day repeated dose toxicity study with Crl:CD(Sprague-Dawley)IGS BR rats, Chengelis (2001), as cited by ECB, 2008, EFSA CONTAM Panel, 2011a) administered 0 or 1,000 mg/kg bw per day of technical HBCDD. The authors found that the concentrations in adipose tissue of the α-stereoisomer were higher than those of the β- and γ-stereoisomers throughout the study period.

In a 28-day oral dose toxicity study, van der Ven et al. (2006) administered to male and female rats 0, 0.3, 1, 3, 10, 30, 100 or 200 mg/kg bw per day of technical HBCDD (10.3% α-, 8.7% β- and 81.0% γ-HBCDD) by gavage. Only the liver and adipose tissue were analysed at the end of the experiment. The liver concentrations of HBCDDs were higher in females than in males over the entire dose range (on average about five times). In adipose tissue, these levels were 2.4 and 1.4 times higher than in liver fat, in females and males, respectively. The authors analysed the HBCDD partitions (ratio γ-/α-HBCDD) in the liver, and showed it was dose dependent: the average ratio was 4.2, 4.9, 4.0, 2.2, 3.1, 0.6 and 0.4 in females, and 2.3, 1.7, 3.0, 2.0, 1.5, 1.5 and 0.9 in males according to the increased the dose.

van der Ven et al. (2009) performed a one-generation reproduction study in Wistar rats where technical HBCDD (10.3% α-, 8.7% β- and 81.0% γ-HBCDD) was administered via the diet at different concentrations of 0, 0.1, 0.3, 1, 3, 10, 30 or 100 mg/kg bw per day. Parental male rats were exposed 10 weeks whereas females were exposed for 8 weeks (starting 2 weeks prior to mating). The authors analysed the HBCDD partitions (ratio γ-/α-HBCDD) in the liver, and showed that the ratio γ/α was 2.2, 1.4, 0.9 and 0.4 in females (for dose between 1 and 100 mg/kg bw per day), and 1.4, 0.8 and 0.2 in males (for doses between 10 and 100 mg/kg bw per day).

3.1.1.1.3. Metabolism

Mice

Hakk et al. (2012) studied the metabolism of α- and γ-HBCDD in adult C57BL/mice. Six female mice were exposed to a single oral dose of 3 mg/kg bw (α- or γ-[^{14}C]-HBCDD). Collection of urine and faeces was done during four consecutive days, and an additional group was sacrificed 3 h post-exposure in order to measure metabolites at tissue levels. The authors found metabolites in liver,

blood, fat, brain, bile, urine and faeces. For α-HBCDD, the authors found four monohydroxylated metabolites in faeces and one of them in liver, brain and adipose tissue. For γ-HBCDD, the authors found one monohydroxylated and three dihydroxylated metabolites in faeces. The monohydroxylated metabolite was also measured in liver and adipose tissue. Hakk et al. (2012) hypothesised that deiodinase may be involved in the debromination of HBCCDs in mice. The authors performed enzymatic hydrolysis of urine and did not detect the presence of glucuronic acid or sulfate conjugates in urine.

In female mice, Szabo et al. (2011a) found different polar metabolites of α-HBCDD at high levels in the liver (38% of the dose applied) and faeces (66% of the dose applied). These metabolites were also observed in the blood and bile.

Szabo et al. (2010) in their study in mice exposed to γ-HBCDD, detected metabolites in the liver, blood, bile, urine and faeces between 3 and 24 h after exposure, but did not identify them.

Sanders et al. (2013) demonstrated that β-[^{14}C]-HBCDD was metabolised following oral and i.v. administration of 3 mg/kg. The authors quantified the radioactivity in urine, tissue and faeces extracts. Although metabolites were not identified, the authors found that most of the β-[^{14}C]-HBCDD-metabolites were excreted in urine (hydrophilic metabolites) and that 30% of the β-[^{14}C]-HBCDD-metabolites in the faeces were non-extractable.

Rats

Laurence et al. (2010) used incubations of α-, β- and γ-HBCDD with liver microsomes from phenobarbital treated rats. The authors described the formation of hydroxy-HBCDD metabolites, but without characterisation. In an additional experiment, the authors examined the reductive debromination of α- and γ-HBCDD. After incubation with dithiothreitol (DTT), used for debromination, the authors did not find debrominated metabolites.

Roosens et al. (2011) incubated α-, β- and γ-HBCDD with liver microsomes from phenobarbital treated rats. The authors detected three types of metabolites: the major ones were hydroxy metabolites of HBCDDs, followed by a hydroxyl-debrominated metabolite (pentabromo) and to a minor extent a debrominated (pentabromo) metabolite.

Esslinger et al. (2011) studied the metabolism of α-, β- and γ-HBCDD in rat liver microsomes. They reported several hydroxylated metabolites of HBCDDs but no debrominated metabolites.

In a similar experiment, following incubation of α-, β- and γ-HBCDD with rat liver microsomes, Abdallah et al. (2014) identified four monohydroxylated and two dihydroxylated metabolites. The authors suggested that metabolism of HBCDDs was mediated by enzymes of the CYP450 system, and that these are important during the stereoselective phase I oxidative metabolism of HBCDDs (in rat).

In Wistar rats exposed to 30 and 100 mg technical HBCDD/kg bw per day for 28 days, several metabolites were identified including four different types of hydroxylated HBCDD metabolites in adipose tissue, liver, lung and muscle (Brandsma et al., 2009). The authors stated that oxidation and reductive debromination were common metabolic routes for HBCDDs, although it was not possible to determine whether individual stereoisomers underwent the same metabolism.

In male Sprague-Dawley rats dosed orally with 3 mg/kg bw of α-, β- and γ-HBCDD, Hakk (2016) quantified several metabolites in urine, faeces, adipose tissue, brain, liver and gastrointestinal tract. The authors showed that α-, β- and γ-HBCDD were metabolised extensively (51%, 65% and 80%, respectively) via different pathways. α-HBCDD was metabolised into two monohydroxylated metabolites, while β- and γ-HBCDD were metabolised via different pathways (stereo isomerisation, oxidation, dehydrogenation, reductive debromination and ring opening).

Bio-isomerisation of HBCDDs

Some authors have observed high concentration of α-HBCDD after a long period in adipose tissue in rainbow trout and in rat (Law et al., 2006), and suggested a bio-isomerisation from β- and γ-HBCDD to α-HBCDD (Szabo et al., 2010). In one study, bio-isomerisation of β- to γ-HBCDD was observed (Sanders et al., 2013).

3.1.1.1.4. Elimination

Mice

a-HBCDD

The elimination of α-[^{14}C]-HBCDD-derived radioactivity after oral administration (3, 10, 30 and 100 mg/kg bw) was mainly in the faeces (~ 40% of the administered dose) and to a lesser extent in the

urine (~ 30% of the administered dose). As shown by the authors, more than 50% of the cumulative elimination of α-HBCDD radioactivity following a single oral dose was excreted via faeces and urine during the first 24 h (Szabo et al., 2011a). The authors also indicated that 66% of the eliminated radioactivity (in urine and faeces) was α-HBCDD derived metabolites (after the first day).

After administration of 3 mg/kg bw, the authors studied the tissue distribution and elimination over 14 days. By measuring concentrations in tissues, the authors showed a biphasic profile of elimination with an initial steep decline (initial half-life or alpha phase) followed by a second steep (terminal half-life or beta phase) (Szabo et al., 2011a) (see **Table 4**).

Table 4: Estimated tissue half-lives (in days) of α-HBCDD in mice (from Szabo et al., 2011a)

Tissue	Half-lives (days)	
	Initial[(a)]	Terminal[(a)]
Liver	0.4 ± 0.1	3.0 ± 0.7
Blood	0.1 ± 0.0	0.5 ± 0.2
Lung	0.3 ± 0.1	15 ± 12.1
Kidney	0.2 ± 0.0	2.1 ± 0.4
Muscle	0.3 ± 0.1	8.0 ± 5.0
Brain	0.1 ± 0.6	3.0 ± 2.0
Fat	—	17 ± 6.4

(a): ± standard deviation considering number of animals = 4–8.

Neonatal mice

Szabo et al. (2011b) investigated the distribution and elimination of α- and γ-HBCDDs in 10-day-old mice and compared these neonatal levels to adult levels previously reported (Szabo et al., 2010, 2011a). According to the authors, 33% of the dose remained in the body in the pups, as compared to 25% in adult mice, 24 h after oral exposure to α-[^{14}C]-HBCDD.

β-HBCDD

Sanders et al. (2013) showed that approximately 90% of the 3 mg/kg bw administered dose of β-HBCDD was excreted in urine and faeces within 24 h of treatment (primarily as β-HBCDD derived metabolites). At higher doses (30 and 100 mg/kg), the elimination of β-[14C]-HBCDD via urine and faeces was less than at the lowest dose. Although the majority of the β-[^{14}C]-HBCDD was excreted within 48 h, the authors found measurable amounts of radioactivity in tissues 4 days post administration.

After administration of 3 mg/kg bw, the authors studied the tissue distribution and elimination over 14 days. By measuring concentrations in tissues, the authors showed a biphasic profile of elimination with an initial steep decline (initial half-life or alpha phase) followed by a second steep (terminal half-life or beta phase) (see **Table 5**) (Sanders et al., 2013).

Sanders et al. (2013) stated that the elimination half-lives of β-[^{14}C]-HBCDD in mice (see **Table 5**) were significantly shorter than for α-HBCDD, but similar to those for γ-HBCDD as calculated by Szabo et al. (2010, 2011a).

Table 5: Estimated tissue half-lives (in days) of β-HBCDD in mice (from Sanders et al., 2013)

Tissue	Half-lives (days)	
	Initial	Terminal
Blood	0.2	2.1
Liver	0.1	1.3
Muscle	0.04	5.3
Adipose tissue	ND	2.5
Brain	0.02	0.2
Lung	0.2	1.5
Skin	0.1	7.0

ND: not detected.

γ-HBCDD

Szabo et al. (2010) showed that the major route of elimination of γ-[^{14}C]-HBCDD was via the faeces in mice after oral exposure. From the analyses of faeces and urine 14 days after the administration, nearly 55% of the dose was excreted in faeces and 30% in urine. As shown by the authors, more than 50% of the cumulative elimination of γ-HBCDD radioactivity following a single oral dose was excreted via faeces and urine in the first 24 h. The authors indicated that 95% of the radioactivity that was eliminated, were γ-HBCDD-derived metabolites (after the first day).

After administration of 3 mg/kg bw, the authors studied the tissue distribution and elimination over 14 days. By measuring concentrations in tissues, the authors showed a biphasic profile of elimination with an initial steep decline (initial half-life or alpha phase) followed by a second steep (terminal half-life or beta phase) (see **Table 6**) (Szabo et al., 2010).

Table 6: Estimated tissue half-lives (in days) of γ-HBCDD in mice (from Szabo et al., 2010)

Tissue	Half-lives (days)	
	Initial	Terminal
Liver	0.3 ± 0.0	2.3 ± 0.2
Blood	0.3 ± 0.0	3.5 ± 0.3
Lung	0.4 ± 0.1	2.3 ± 0.2
Kidney	0.2 ± 0.0	2.8 ± 0.2
Muscle	1.0 ± 0.1	3.6 ± 0.3
Skin	0.4 ± 0.0	5.2 ± 0.3
Brain	0.1 ± 0.0	0.8 ± 0.1
Fat	0.9 ± 0.1	3.6 ± 0.2

Szabo et al. (2011b) investigated the distribution and elimination of α- and γ-HBCDDs in 10-day-old mice and compared these neonatal levels to adult levels previously reported (Szabo et al., 2010, 2011a). According to the authors, 16% of the dose remained in the body in the pups, as compared to only 2% in adult mice, 24 h after oral exposure to γ-[^{14}C]-HBCDD.

Rats

Hakk (2016) showed after an oral dose of 3 mg/kg bw HBCDDs (α-, β- and γ-) that faeces were the major route of excretion, cumulatively accounting for 42% of the dose for α-HBCDD, 59% for β-HBCDD and 53% for γ-HBCDD after 96 h. In urine, the cumulative excretion accounted for 13% of the dose for α-HBCDD, 30% for β-HBCDD and 21% for γ-HBCDD after 96 h.

Elimination was slower in rats than in mice (Szabo et al., 2010, 2011b; Sanders et al., 2013).

The elimination rates in urine and faeces show that α-HBCDD has a higher propensity to accumulate than β- and γ-HBCDD.

Hakk (2016) calculated elimination half-lives in rat based on urine/faeces elimination, for α-HBCDD, β-HBCDD and γ-HBCDD: the corresponding values were 3.3 ± 0.7 days, 1.1 ± 0.2 days and 2.0 ± 0.3 days, respectively.

3.1.1.1.5. Summary on toxicokinetic studies in rodents

Results from mice and rat studies showed that the absorption from the gastrointestinal tract was rapid with an oral absorption for HBCDDs after oral gavage around 85–90% of the total dose (by comparing kinetics and disposition between i.v. and oral routes of exposure).

HBCDDs are distributed to a number of tissues, including adipose tissue, muscle and the liver. Elimination of HBCDDs-derived radioactivity is predominantly via faeces, but it is also eliminated via urine. In mice, the studies suggest that more than 66%, 90% and 95% of the radioactivity excreted from the body correspond to different metabolites of α-, β- and γ-HBCDD, respectively.

γ-HBCDD was more rapidly metabolised and eliminated than α-HBCDD, with a terminal half-life in mice of 4 days (Szabo et al., 2010). α-HBCDD was more biologically persistent due to a lower metabolism with a terminal half-life of 17 days (in adipose tissue). Some authors have suggested that debromination could be a pathway of HBCDD metabolism: in mice and rat, metabolic debromination and hydroxylation of HBCDDs. Conversion of γ-HBCDD to α- and β-HBCDD have been reported. No stereoisomerisation of α-HBCDD was reported.

After oral administration in mice, elimination of α-[^{14}C]-HBCDD and γ-[^{14}C]-HBCDD are mainly in the faeces (40% and 55% of the administered dose) and in the urine (both 30% of the administered dose). β-[^{14}C]-HBCDD was equally excreted in urine and faeces within 24 h of treatment.

Predominance of α-HBCDD in tissue could be explained by either a difference in toxicokinetics, where γ-HBCDD is metabolised and eliminated at a more rapid rate than α-HBCDD, and/or stereoisomerisation of γ-HBCDD to α-HBCDD.

3.1.1.2. Toxicokinetic studies in humans

3.1.1.2.1. Absorption

No *in vivo* data were identified.

Abdallah et al. (2012) showed different bioaccessibility values among HBCDD stereoisomers present in dust. From an *in vitro* test system including human gastrointestinal tract parameters, they reported 92%, 80% and 72% of bioaccessibility for α-, β- and γ-HBCDD, respectively. The bioaccessibility was calculated from the percentage of the average mass of the three HBCDD stereoisomers in the supernatant of the test system to the average mass of the HBCDD stereoisomers present in the extracted dust. It was suggested that this may be attributed to the lower aqueous solubility of the γ-isomer (2 μg/L) compared to the α- and β-isomers (45 and 15 μg/L, respectively). No significant change in the enantiomeric fractions of HBCDDs was observed in any of the studied samples.

3.1.1.2.2. Distribution

HBCDDs have been detected in human adipose tissue, breast milk, placenta and blood, as well as in hair and faeces (see **Section 3.1.1.4**).

3.1.1.2.3. Metabolism

Erratico et al. (2016) suggested that CYP3A4 was involved in the *in vitro* metabolism of the HBCDD stereoisomers. After incubation of human liver microsomes with racemic mixtures of α-, β- and γ-HBCDD, the authors found formation of several hydroxylated metabolites.

Abdallah et al. (2015) performed an additional metabolic study with human hepatocytes incubated during 24 h with α-, β- and γ-HBCDD. Chromatographic analysis revealed phase I oxidative metabolism of HBCDDs (hydroxylated metabolites) and indicated a reductive debromination by the detection of penta- and tetrabrominated metabolites. They also identified glucuronide metabolites of HBCDDs.

3.1.1.2.4. Elimination

In a biomonitoring study in toddlers, Sahlström et al. (2015a) detected the presence of α-, β and γ-HBCDD in the faeces. However, this study did not distinguish between faecal excretion following biliary excretion or direct excretion (non-absorption by the gastrointestinal tract).

Geyer et al. (2004, extended abstract) estimated a terminal elimination half-life for HBCDDs of 64 days (range: 23–219 days) in humans using a linear one-compartment open toxicokinetic model based on body burden and daily intake in humans of 142 ng per day. The authors estimated this daily intake and the total body burden in non-occupationally exposed adult humans from a Swedish market basket study. Assuming a bioavailability of 100%, the corresponding lipid mass would be 13.5 kg for males and 18.7 kg for females. According to the authors, the large range observed in half-lives could be due to the difference of fat mass in humans that would affect bioaccumulation and elimination. The CONTAM Panel noted that the method to estimate the half-life is not fully described in the extended abstract and this leads to uncertainties regarding the half-life calculation (see **Section 3.5.4**).

Abdallah and Harrad (2011) compared the concentrations measured in human milk samples with the estimated intakes of HBCDDs using a simple one-compartment model. They obtained good agreement between the predicted and the observed body burdens of the three HBCDD stereoisomers by using a half-life for α-HBCDD in humans of 165 days and a half-life of 55 days for the β- and γ-isomers. For α-HBCDD, this is representing 75% of the maximum half-life of 219 days estimated previously by Geyer et al. (2004, extended abstract), and for the β- and γ-isomers 25% of the maximum half-life of 219 days.

3.1.1.2.5. Summary on toxicokinetic studies in humans

Limited data are available on the toxicokinetics of HBCDDs in humans. As there is no *in vivo* data, an estimate of the oral absorption of HBCDDs cannot be made. Biomonitoring studies report that HBCDDs have been detected in different human fluids (blood, breast milk, faeces) and tissues (adipose, placenta, hair).

In vitro studies have suggested that CYP3A4 may be involved in metabolism of the HBCDD stereoisomers based on formation of several hydroxylated metabolites. There is also *in vitro* evidence of glucuronide formation.

Only one study provided half-life values in humans with a value of 64 days (range of 23–219 days).

3.1.1.3. Transfer of HBCDDs from feed to food-producing animals

Several studies have been identified on the transfer of HBCDDs from feed into several farm animals, i.e. laying hens, broilers, ducks, pigs and fish.

3.1.1.3.1. Laying hens, broilers and ducks

Fournier et al. (2012) studied the fate of ingested HBCDDs in laying hens. The authors fed 48 laying hens with HBCDDs at 1.1 ng/g diet (containing 0.1%, 0.2% and 99.7% of α-, β- and γ-HBCDD, respectively) for 21 days and analysed the three stereoisomers in egg yolk, abdominal fat and liver after 1, 4, 8, 11, 16, 21, 22, 24, 29 or 39 days. In the control hens, α-HBCDD was quantified in abdominal fat (32–33 pg/g lipid weight at days 0 and 39, respectively) and in liver (51–69 pg/g lipid weight at days 0 and 39, respectively). In the treated group, concentrations of α- and γ-HBCDD in egg yolk, abdominal fat and liver varied between days and between tissues. During the exposure period, α-HBCDD concentrations varied in egg yolk (between 23 and 380 pg/g lipid weight), in abdominal fat (between 25 and 108 pg/g lipid weight) and in liver (between 150 and 235 pg/g lipid weight). γ-HBCDD concentrations varied in abdominal fat (between 23 and 490 pg/g lipid weight) and in liver (between 114 and 273 pg/g lipid weight). In the treated group, at day 39, α-HBCDD concentrations of 80, 102 and 3.5 pg/g lipid weight were quantified in abdominal fat, liver and yolk, respectively. γ-HBCDD was not detected in egg yolk nor in liver of the control group, but concentrations of 17–21 pg/g lipid weight were quantified in abdominal fat. In comparison, at 39 days, these concentrations were 33, 69 and 3.5 pg/g lipid weight in the control group. The authors estimated a transfer rate of 1.2% from ingested γ-HBCDD to egg yolk (at steady state). Over the 21-day exposure period, the proportions of ingested γ-HBCDD stored in abdominal fat and in liver were 0.74% and 0.025%, respectively. Despite the very low level of α-HBCDD in the feed (0.1% of the 1.1 ng/g), α-HBCDD was found in egg yolk, abdominal fat and liver of the hens. The authors suggested isomerisation of γ- to α-HBCDD in laying hens. According to the authors, over the 21-day exposure period, 0.17% of the ingested γ-HBCDD was excreted in egg yolk (as α-HBCDD) and 0.17% and 0.025% were measured in abdominal fat and in liver, respectively.

Jondreville et al. (2017a) studied the elimination and accumulation of α-HBCDD in broilers. The authors exposed 29 fast-growing and 50 slow-growing chickens to α-HBCDD at 50 ng/g feed over 42 and 84 days. An additional 10 animals for each group were used as control. The authors showed that the concentrations of α-HBCDD in leg, breast, liver and abdominal fat were higher in slow-growing than in fast-growing strains. In both strains, the concentration of α-HBCDD was higher in abdominal fat than in other tissues. The calculated bioaccumulation factors (i.e. the ratio of the concentration of α-HBCDD in the tissue (lipid based) to its concentration in feed, calculated at market age (6 and 12 weeks in fast-growing and slow-growing broilers, respectively), were 5.2 and 6.7 in leg, 2.2 and 2.1 in breast muscle, 8.4 and 8.9 in abdominal fat, in fast- and slow-growing chicken, respectively. In slow-growing chickens, the authors estimated half-life values of 53, 24 and 47 days in leg, muscle and abdominal fat, respectively. In a previous work of this group, Domínguez-Romero et al. (2016) demonstrated that after exposure to α-HBCDD (50 and 5 ng/g feed) for 18 or 11 days, α-HBCDD was transferred to eggs and significantly accumulated in adipose tissue. The calculated bioaccumulation factors were 5.2, 3.6 and 9.2 in eggs, liver and abdominal fat, respectively.

Zheng et al. (2017) measured HBCDDs in diet sources and in chicken tissues in adult (n = 12) and hatchling chicken (n = 9). In feed samples, the highest HBCDD concentration measured was 0.69 ng/g dw while the highest concentration in soil was 28.8 ng/g dw. For adult chickens, the order of α-HBCDD concentrations within the tissues studied was: gonad > visceral fat > pectoral muscle > intestine > stomach > kidney > lung > serum > liver. The authors did not detect α-HBCDD in brain. For hatchling chicken, the order of α-HBCDD concentrations was: pectoral muscle > liver (only 2 tissues studied).

Xia et al. (2018) studied the accumulation of HBCDDs in ducks exposed at 0 (n = 8, control group with blank capsule), 0.8 mg/kg bw (n = 32) and 1.6 mg/kg bw per day (n = 32). At 0, 7, 14 and 21 days after exposure, eight ducks from each group were sacrificed. After a 21-day depuration period, the order of HBCDD concentrations (in ww) at 0.8 mg/kg bw per day within the tissues studied was: skin > tongue > intestines > heart > gizzard > muscle > liver > lung > brain > blood. At 1.6 mg/kg per day, the order of HBCDD concentrations (in ww) within the tissues studied was: skin > tongue >

intestines > gizzard > muscle > liver> heart > lung > brain > blood. The high levels in skin and tongue were probably attributed to the high-fat content. The authors calculated half-lives for the different tissues, and these varied between 17–70 days for α-HBCDD, 3.8–9.1 days for β-HBCDD and 8.8–15 days for γ-HBCDD.

3.1.1.3.2. Pigs

Royer et al. (2017) performed a study in order to establish a physiologically based pharmacokinetic model for α-HBCDD in pigs. The authors exposed 56 pigs during 19, 49, 70, 91 and 112 days to α-HBCDD at 0, 32 or 297 μg/kg feed (not lipid based). Two control pigs were sacrificed at day 1, and three pigs per treatment were sacrificed at 20, 50, 71, 92 and 113 days. The liver, dorsal fat and semi-membranous muscle were collected. The authors found that α-HBCDD concentrations were higher in adipose tissue than in muscle and were lower in the liver. The authors estimated accumulation ratios for α-HBCDD of 6.6 and 5.3, respectively, in dorsal fat and semi-membranous muscle. The accumulation ratio for liver was not estimated.

3.1.1.3.3. Fish

Berntssen et al. (2011) developed a toxicokinetic model in Atlantic salmon. For this purpose, the authors exposed Atlantic salmon to α-HBCDD at 280 μg/kg feed per day for 2 months, followed by a depuration period of 3 months. Three fish were sacrificed on day 0, 8, 14, 26, 41 and 54 of the uptake period and on day 0, 1, 3, 5, 7, 14, 28, 42, 55 and 83 of the exposure period. Based on the fat storage in the fillet for α-HBCDD, the authors showed that their model simulated a concentration of α-HBCDD in the range of 0.2–1.8 μg/kg with a low background and high levels of α-HBCDD in the feed at 600 days.

Esslinger et al. (2010) exposed mirror carps (n = 56 per group) to racemic γ-HBCDD in order to study potential bioisomerisation. The commercial fish feed contained a concentration of 26.0 ± 1.6 ng/g of (+)-γ-HBCDD and 16.8 ± 0.4 ng/g of (-)-γ-HBCDD. After treatment for 14 days, an increase of γ-HBCDD was measured in the fillet (for both racemic) compared to the control group. The authors did not observe bioisomerisation of γ-HBCDD in this species.

Zhang et al. (2014) studied the accumulation of α-, β- and γ-HBCDD in mirror carps (n = 40 fish per group) over 30 days followed by a depuration period of 30 days. The authors detected the three HBCDD stereoisomers in different tissues (gills, viscera, muscle and skin) of the mirror carp with an increased concentration over the 30 days. The authors calculated kinetic bioconcentration factors (BCFs). For α-HBCDD the BCFs were 8.58×10^3, 1.15×10^4, 5.57×10^3 and 6.40×10^3 in gills, viscera, muscle and skin, respectively. For β-HBCDD, the BCFs were 322, 642, 187 and 204, and for γ-HBCDD were 237, 584, 221 and 227, respectively. The accumulation for α-HBCDD was higher than for the β- and γ-stereoisomers. At the end of the depuration period, the authors described transformation of β- and γ-isomers into α-HBCDD (53.0–92.9% for β-HBCDD and 96.2–98.6% for γ-HBCDD).

3.1.1.3.4. Toxicokinetic models for the transfer from feed to food-producing animals

Meda et al. (2017, 2020) described generic PBPK models for broilers and pigs. These models have been validated using data from Jondreville et al. (2017a) (for broilers) and data from Royer et al. (2017) for pig. These models were calibrated to take into account differences in lipid content according to species, and validated using experimental data where animals were exposed to HBCDDs.

3.1.1.3.5. Summary on the transfer from feed to food of animal origin

In broilers, α- and γ-HBCDD have been measured in egg yolk, abdominal fat and liver (lipid based). Isomerisation of γ- to α-HBCDD in laying hens has been suggested by some authors. In ducks, accumulation of HBCDDs has been described, with high levels in skin and tongue, probably attributed to the high-fat content.

In pigs, HBCDD concentrations seemed to be higher in the adipose tissue than in the muscle and in the liver.

In fish, HBCDDs have been also detected in tissue with high-fat content.

In summary, from the few available studies on the transfer of HBCDDs from feed to food of animal origin, accumulation of HBCDDs mainly happens in adipose tissue among laying hens, broilers, ducks (organ with high-fat content), pigs and fish. In the laying hens, transfer to eggs was also described.

Some toxicokinetic models have been constructed based on data obtained in broilers and pigs. These models could be used to assess the risk of meat contamination by HBCDD in animals.

3.1.1.4. Levels in human tissues

3.1.1.4.1. Human milk

The previous EFSA Opinion on HBCDDs (EFSA CONTAM Panel, 2011a) summarised the occurrence data in human milk published in literature until 2010. Except for one study, the levels ranged between 0.13 and 31 ng/g lipid with mean and/or median values generally below 2 ng/g lipid.

Table 7 summarises the occurrence data on HBCDDs in human milk from European mothers published in the open domain since the previous EFSA Opinion on HBCDDs. In case of analysis by GC-based methods for which a separation of the diastereomers is not possible, the results are given as Total HBCDDs. Although the data were published between 2011 and 2020, most of the samples were collected between 2000 and 2010. In general, the results are in a comparable range with the data reported in the previous EFSA Opinion. This holds also true for the share of α-HBCDD which generally contributes more than 75% to the sum of HBCDDs.

It is noteworthy that Abdallah and Harrad (2011) also detected the degradation products pentabromocyclododecenes (average = 0.04 ng/g lipid; n = 9) and tetrabromocyclododecadienes (average = 0.15 ng/g lipid; n = 25) in some samples. Moreover, they reported on the HBCDD enantiomer profiles in the analysed samples. While the enantiomer fractions (EFs) of β-HBCDD and γ-HBCDD showed no significant deviations from racemic (average EF of 0.49 and 0.51, respectively), substantial enrichment of the (−)-α-HBCDD enantiomer was evident (EF = 0.29). Similar results have been reported by Eljarrat et al. (2009).

Harrad and Abdallah (2015) measured concentrations of HBCDDs and the HBCDD degradation products tetrabromocyclododecadienes along with PBDEs, and TTBPA in human milk collected in 2010–2011 from 10 first-time mothers from the UK each over the first 12 months post-partum, amounting to 120 samples overall. While for HBCDDs the correlation analysis revealed no statistically significant change in concentration with time over the 12 months lactation period covered, the concentration of the sum of tetrabromocyclododecadienes showed a significant increase with 1.4% per month ($p = 0.02$).

Antignac et al. (2016) analysed human milk samples from French, Danish and Finnish women for a number of POPs, including α-HBCDD. A statistical evaluation of the results showed no significant correlations between PBDEs and HBCDD, and between the age of the mother and the respective HBCDD content.

Thomsen et al. (2010) studied elimination rates of HBCDDs and other persistent organic pollutants in nine Norwegian single-child primiparous mothers and one mother breast-feeding her second child by collecting breast milk samples (n = 70) monthly from about 2 weeks to up to 12 months after birth. The number of samples collected by each mother was 3–10. While three mothers sampled breast milk during 2001 and 2003, six mothers sampled during 2005 and 2006 and one in 2008 and 2009. Total HBCDDs could be detected in 32 samples with a median of 0.27 ng/g lipid (range: < 0.2–3.0 ng/g lipid). No clear time trend during the lactation periods could be observed as the HBCDD levels showed large variations and the concentrations were below LOQ in several samples.

Tao et al. (2017) analysed 10 human milk samples collected in 2014–2015 in the UK and compared the results with the data reported by Abdallah and Harrad (2011). No statistically significant change in the HBCDD levels in human milk collected between 2014 and 2015 and 2010 was observed.

Croes et al. (2012) analysed a human milk pool consisting of 84 individual samples collected in 2009–2010 in a rural area in Belgium and found a 153% higher level compared to the pooled Belgium sample analysed in 2006 within the World health Organisation (WHO) field study.

Sahlström et al. (2015b) analysed two human milk pools collected from 30 mothers each in 2009 and 2010, respectively, within a study where they compared estimated intakes of HBCDDs and other BFRs via diet and dust to internal concentrations in a Swedish mother–toddler cohort. Levels for α-, β- and γ-HBCDD as well as for ΣHBCDDs (sum of α-, β- and γ-HBCDD) were given as 8.4, < 0.18, < 1.3 and 9.4 pg/g ww, respectively. Considering the mean lipid content of 3%, the results are below 0.5 ng/g lipid and thus are at the lower end of reported occurrence data in human milk. The concentrations in both pools were similar.

Lignell et al. (2014) determined several POPs including Total HBCDDs in human milk collected 2012 from 30 randomly recruited primiparas from Uppsala (Sweden) and compared the results with respective concentrations in earlier surveys. The temporal trend analysis included results from samples collected in 2002–2004 and 2009–2012. The trend for Total HBCDDs is reported to be uncertain and showed a non-significant decrease of 2.5% per year during the period 2002–2012.

In a follow-up study, Gyllenhammar et al. (2017) analysed 30 human milk samples collected 2015–2016 from Swedish mothers living in Uppsala for Total HBCDDs among other POPs. The results

enabled an updated trend analysis. For Total HBCDDs, the authors reported a significant downward trend for the whole study period (1996–2016) with a declining rate of 2.0% per year. A significant change point was observed around year 2002–2003 with an increasing trend before that year and a decreasing trend after.

Wemken et al. (2020) analysed 16 pools from 92 Irish primiparas for α-, β- and γ-HBCDD, 8 PBDEs and decabromodiphenyl ethane (DBDPE). Human milk sampling and donor recruitment were comparable with the previous study of Pratt et al. (2013) in order to facilitate elucidation of possible time trends for BFRs in human milk from Irish women. Using a t-test, the authors compared the concentrations of ΣHBCDDs in individual pools of the two studies and reported that the current concentrations are significantly lower ($p < 0.05$) than in samples analysed by Pratt et al. (2013).

Based on scientific literature published between 1995 and 2011, Fång et al. (2015) performed a global review on spatial and temporal trends of the Stockholm Convention POPs, including HBCDDs in human milk. The levels reported for HBCDDs human milk collected in Africa and Asia are generally in the same range as the occurrence data analysed in samples from Europe at that time. The samples from Japan (collected between 1987 and 2007) and from Sweden (collected between 1987 and 2010) were reported to show increasing trends over the whole period of 5.4% per year ($p < 0.061$) and 7.6% per year ($p < 0.001$), respectively.

In their review, Shi et al. (2018) summarised data on HBCDDs in human milk from China and compared the results with levels found in other regions of the world. A substantial increase of HBCDD levels in human milk from China collected in 2007 and in 2011 was observed. While in 24 pooled human milk samples from 12 provinces collected in 2007, the HBCDD concentrations ranged from < LOQ to 2.78 ng/g lipid, the respective levels in 29 pools from 16 provinces sampled in 2011 amounted to 1.02–81.1 ng/g lipid, with mean and median levels of 10.1 and 6.83 ng/g lipid, respectively. Similar levels were found in 103 individual human milk samples collected in 2011 in Beijing with mean concentration of 4.29 ng/g lipid (range: < LOQ–78.3 ng/g lipid). A comparison of the HBCDD levels with human milk analysed in other countries revealed that the contamination of human milk from China is higher than from most other regions worldwide.

Huang et al. (2020) analysed 111 human milk samples collected in 2014 from 27 Beijing mothers for HBCDDs and TBBPA, whereby each mother provided one sample per month for 3 months. All mothers were primiparous with an average age of 29.7 years. The ΣHBCDDs (sum of α-, β- and γ-HBCDD) ranged from < LOD to 36.3 ng/g lipid, with mean and median concentrations of 7.58 and 5.67 ng/g lipid, respectively. Compared to the levels analysed in 2011, a statistically significant increase in HBCDD levels was observed. A correlation analysis revealed no significant trend in concentrations over the 3 months of lactation.

Since 1987, the WHO (partly in cooperation with the United Nations Environment Programme (UNEP)) conducted several coordinated surveys on the occurrence of various POPs in human milk. These surveys were not primarily intended to compare levels of POPs among countries, but rather to examine levels within countries over time. Therefore, strict protocols had to be followed with respect *inter alia* to selection of donors, location and time of sampling, storage and pooling of samples (generally 1 pool consists of 50 individual samples) and shipping of the samples to the laboratory (WHO, 2007). To ensure consistency in the analytical measurements, from the third round, all samples were analysed by one laboratory, i.e. the EURL for Halogenated POPs in Feed and Food in Freiburg/ Germany. The latest results of these surveys on HBCDDs in European pooled human milk samples are given in **Table 8** (Malisch and Schächtele, 2019, see Documentation provided to EFSA). The table shows the levels of α-, β- and γ-HBCDD as well as the sum of the three isomers (ΣHBCDDs) as upper bound (UB) and lower bound (LB) values, respectively, given as ng/g lipid.[27] The last two columns present the ratio of the predominant stereoisomer α-HBCDD in relation to the sum of HBCDDs, calculated as UB and LB, respectively.

Considering the most recent occurrence data generated as part of the WHO/UNEP coordinated human milk studies since the last EFSA Opinion on HBCDDs (EFSA CONTAM Panel, 2011a), collected and analysed between 2014 and 2016, the UB and LB levels for the sum of the three stereoisomers range from 0.70–16.1 and 0.00–16.0 ng/g lipid, respectively. The UB and LB medians for the sum of the three stereoisomers are 3.12 and 3.0 ng/g lipid, respectively. As indicated in **Table 8**, α-HBCDD is the predominant stereoisomer and generally contributes to more than 75% to the sum of HBCDDs.

[27] In literature, both terms 'lipid' and 'fat' are cited, generally without stating which fractions or constituents were actually measured. Therefore, in this Opinion, both terms are considered synonymous, unless otherwise stated.

In two cases, human milk pools were collected from the same country in 2009 and 2014, and 2009 and 2015, respectively. It is noteworthy that the levels for HBCDDs in the recent samples are substantially higher than in the earlier samples. However, the low number of samples does not allow deriving a general time trend from this observation.

Table 7: HBCDDs in human milk from European mothers published in the open domain since the previous EFSA Opinion on HBCDDs

Country	Year of sampling	n	α-HBCDD	β-HBCDD	γ-HBCDD	ΣHBCDD	Total HBCDDs	Reference
			Mean (median), range					
France	2010	106	< LOD[(a)]–23.6	< LOD–6.6	< LOD–6.4	2.7–33.2	–	Inthavong et al. (2017)
France	2011–2014	41	(0.56), 0.22–4.21	–	–	–	–	Antignac et al. (2016)
Denmark	1997–2002	435	(0.31), 0.02–28.71	–	–	–	–	Antignac et al. (2016)
Finland	1997–2002	22	(0.31), 0.03–2.19	–	–	–	–	Antignac et al. (2016)
UK	2010	34	4.91 (3.17), 0.75–19.71	0.32 (0.30), 0.08–0.75	0.73 (0.56), 0.13–2.29	5.95 (3.83), 1.04–22.37	–	Abdallah and Harrad (2011)
UK	2010–2011	120[(b)]	5.27	0.48	0.79	–	–	Harrad and Abdallah (2015)
UK	2014–2015	10	–	–	–	3.2 (2.9), 0.69 –7.1	–	Tao et al. (2017)
Ireland	2010	11[(c)]	2.59, (1.50–3.44)	0.46, (0.29–1.56)	0.47, (0.29–0.94)	3.52, (1.67–5.94)	–	Pratt et al. (2013)
Ireland	2016–2018	16[(d)]	1.5 (1.7), 0.66–3.0	0.22 (0.34), < 0.05–0.53	0.27 (0.21), < 0.05–1.5	2.0 (1.8), 0.83–3.6	–	Wemken et al. (2020)
Czech Republic	2010	50	< 1–76[(e)]	–	–	–	–	Lankova et al. (2013)
Belgium	2009–2010	84[(f)]	3.20	0.05	0.55	3.80	–	Croes et al. (2012)
Sweden	2008	18	–	–	–	–	< 0.32–1.5	Björklund et al. (2012)
Sweden – Uppsala – Gothenburg – Lund – Lycksele	2000–2004	92 37 36 39	–	–	–	–	0.3 (< 0.2–4.4) < 0.4 (< 0.4–2.4) 0.4 (< 0.2–5.9) 0.4 (0.09–10)	Glynn et al. (2011)
Sweden	2012	30					0.40 (0.33), < 0.20–1.5	Lignell et al. (2014)
Sweden	2015–2016	30					0.18 (0.15), < 0.09–0.72)	Gyllenhammar et al. (2017)

Note: All concentration levels are expressed in ng/g lipid.
(a): The LOD and LOQ were reported as 0.5 and 2.5 ng/g lipid for HBCDD diastereomers. α-, β- and γ-HBCDD diastereoisomers were quantified in 8%, 3% and 3% of samples.
(b): 12 samples of each 10 mothers sampled over a period of 12 months.
(c): 11 pools from 109 first-time mothers.
(d): 16 pools from 92 Irish primiparas.
(e): The LOQ for α-HBCDD is given as 1 ng/g lipid and the frequency of detection as less than 30%.
(f): Pool of 84 samples from a rural area in Belgium.

Table 8: HBCDDs in human milk pools from various European countries as analysed in the frame of the WHO/UNEP coordinated studies

Country	Year	Lipid	α-HBCDD	β-HBCDD	γ-HBCDD	ΣHBCDDs LB	ΣHBCDDs UB	Ratio α/Σ LB	Ratio α/Σ UB
		%	ng/g lipid					%	%
Austria	2016	4.1	5.60	< 0.10	< 0.10	5.60	5.80	100	97
Switzerland	2016	4.2	0.50	< 0.10	< 0.10	0.50	0.70	100	71
Belgium	2015	3.6	2.00	< 0.06	< 0.06	2.00	2.12	100	94
Lithuania	2015	3.4	3.00	0.30	< 0.06	4.00	4.06	75	74
Moldova	2015	4.1	8.00	< 0.06	0.20	8.00	8.06	100	99
	2009	4.7	2.84	< 0.10	< 0.10	2.84	3.04	100	93
Netherlands	2014	3.0	0.60	< 0.06	< 0.06	0.60	0.72	100	83
Czech Republic	2014	3.8	1.00	< 0.06	< 0.06	1.00	1.12	100	89
Bulgaria	2014	3.9	2.00	< 0.06	< 0.06	2.00	2.12	100	94
Croatia	2014	4.0	3.00	< 0.06	< 0.06	3.00	3.12	100	96
Georgia	2014	4.0	4.00	< 0.06	< 0.06	4.00	4.12	100	97
	2009	4.8	1.27	< 0.10	< 0.10	1.27	1.47	100	86
Romania	2014	3.7	15.00	< 0.06	0.70	16.0	16.1	94	93
Finland	2007	–	< 0.40	< 0.40	< 0.40	0.00	1.20	–	–
Luxembourg	2006	3.5	1.10	< 0.40	< 0.40	1.10	1.90	100	58
Slovak Republic	2006	3.2	1.80	< 0.20	< 0.20	1.80	2.20	100	82
Hungary	2006	4.1	2.20	< 0.40	< 0.40	2.20	3.00	100	73

LB: lower bound. UB: upper bound.

3.1.1.4.2. Human blood

Recent analyses of human blood for HBCDDs are scarce, especially from European populations. In the previous EFSA assessment, several studies were described reporting levels of HBCDDs in serum and one study in cord blood (EFSA CONTAM Panel, 2011a). The results of the few published studies from Europe after 2010 focus on blood from adults and are summarised below.

Ålander et al. (2019) analysed 57 serum pools including 622 individual samples collected between 1996 and 2017 from first-time mothers in Uppsala (Sweden) for Total HBCDDs among other BFRs. The concentrations for Total HBCDDs were in most cases < LOQ which was around 0.5 ng/g lipid. A statistically significant decreasing trend of 4% per year was reported by the authors for the whole study period.

Jansen et al. (2018) analysed serum samples that were collected between 2012 and 2014 from 63 patients before and 1 year after bariatric surgery for organochlorine pesticides, PCBs and certain BFRs by GC/low resolution MS (LRMS). The LOD for Total HBCDDs was 0.033 ng/g lipid. Only 3% and 5% of the serum samples were above LOD before and 1 year after bariatric surgery, respectively. The Total HBCDD levels for the two time points ranged between < LOD–169 ng/g lipid and < LOD–65.2 ng/g lipid, respectively.

Bjermo et al. (2017) analysed 170 serum samples, collected in 2010–2011 from Swedish adults (age 50 ± 17 years old) for HBCDDs and PBDEs by GC/LRMS. The LOD and limit of quantification (LOQ) for Total HBCDDs were 0.017 and 0.50 ng/g lipid, respectively. The share of samples above LOD and LOQ are reported as 63% and 13%, respectively. The levels of Total HBCDDs in serum ranged from < LOD–77 ng/g lipid. The 95th percentile was 1.8 ng/g lipid. By replacing concentrations below LOD by ½ LOD, the median was calculated as 0.10 ng/g lipid.

Darnerud et al. (2015) analysed 36 pooled serum samples from Swedish first-time mothers for HBCDDs and PBDEs by GC/LRMS. A total of 413 individual serum samples were collected between 1996 and 2010 to prepare the pools. Each pool consisted of 5–25 individual samples (approximately three pools per year). The HBCDD levels seemed to be stable up to about 2005 and thereafter decreased. Considering the entire study period from 1996 to 2010, a significant HBCDD decrease in the pooled serum samples was observed. The change was reported as –6.9% per year ($p = 0.002$). In addition, serum/breast milk correlations for HBCDD and PBDE levels in 30 paired samples from individual mothers sampled in 2010 were studied. As most serum samples from 2010 were below LOQ, a calculation of HBCDD correlations between serum and milk could not be performed.

Sahlström et al. (2014) analysed matched serum samples from 24 Swedish mothers (24–40 years old) and their toddlers (11−15 months of age) for HBCDDs and other BFRs. Samples were collected in 2009–2010. α-, β- and γ-HBCDD were not detected at method LOQs, reported as 0.44, 0.20 and 0.34 pg/serum sample,[28] respectively.

Lignell et al. (2013) investigated correlations between PBDEs and Total HBCDDs in paired blood serum and breast milk samples collected in 2010 from 30 mothers living in Uppsala (Sweden) with the aim to evaluate if the concentration of PBDE/HBCDD in breast milk is a good indicator of maternal body burden and possibly also of prenatal exposure of the infant. While the Total HBCDDs concentrations in breast milk ranged between < 0.13 and 1 ng/g lipid, most of the levels in serum were below the LOQ which impeded the evaluation of correlations.

Fromme et al. (2016) analysed blood samples collected in 2013 in Germany from 42 randomly selected subjects (20–68 years old) for a number of POPs, including HBCDDs. α- and β-HBCDD could only be detected in 3 and 4 samples, respectively, with concentrations between the LOD of 5 ng/g lipid and the LOQ of 16 ng/g lipid. Maximum values were reported as 9 and 15 ng/g lipid, respectively.

Human serum samples (n = 61; age range 20–65 years) were collected in Greece in 2007 and analysed for HBCDDs, PBDEs, PCBs and organochlorine pesticides (Kalantzi et al., 2011). Thirty samples were collected from computer clerks of a large computer company working full-time with computers, and 31 from a control population with no computer use. HBCDDs were detected in 70% of the serum samples. The levels ranged between 0.49 and 38.8 ng/g lipid (median: 1.32; mean: 3.39 ng/g lipid). Differences in HBCDD body burden between the two study groups are not reported.

In summary, the available data on HBCDDs in blood from European populations generally point to low levels with medians around 1 ng/g lipid, however, with some samples containing substantially higher concentrations. This is similar to global human HBCDD levels in blood as shown, e.g. for

[28] The authors report the method LOQs not per gram serum, but individually for each sample with respect to the different sample intakes of 0.5–5 g.

Canada (Rawn et al., 2014a), Korea (Kim and Oh, 2014), China (Shi et al., 2013) and Australia (Drage et al., 2017, 2019).

3.1.1.4.3. Other human tissues

Malarvannan et al. (2013a) analysed HBCDDs in paired visceral fat and subcutaneous abdominal fat samples collected in 2010–2012 from 52 obese individuals in Belgium. Median concentrations of 4.1 ng/g lipid in visceral fat (range: 0.9–53 ng/g lipid) and 3.7 ng/g lipid in subcutaneous fat (range: 0.89–89 ng/g lipid) for ΣHBCDDs (sum of α-, β- and γ-HBCDD) were reported. The share of α-HBCDD to the sum of the three HBCDD stereoisomers was 85% and 97%, respectively. The levels in the two fat depots were not significantly different. The HBCDD concentrations in the fat samples did not correlate with age.

Rawn et al. (2014b) determined HBCDDs in Canadian human fetal liver (n = 52) and placental tissue (n = 142). Tissue samples were collected between 1998 and 2010 following elective pregnancy terminations. While the concentration of ΣHBCDDs in fetal livers ranged from < 1 to 4,500 ng/g lipid, the corresponding concentrations in placenta were determined as < 1–5,600 ng/g lipid. No clear temporal trend was observed in liver samples nor was a significant relationship found between fetal age and ΣHBCDD levels. HBCDD concentrations in liver/placental paired tissue samples did not show a correlation. The results of the study indicate that HBCDDs are already present at measurable concentrations in developing fetuses from as early as 6.5 weeks.

Sahlström et al. (2015a) investigated the feasibility of using faeces as a non-invasive matrix to estimate serum concentrations of BFRs in toddlers for biomonitoring purposes. In their study, faeces samples from 22 toddlers (11–15 months old) were analysed for HBCDDs and other BFRs, and the results were compared with previously analysed matched serum samples. The detection frequency for α-, β- and γ-HBCDD in the faeces was reported to be 100%, 61% and 74%, respectively. The medians for the three stereoisomers were 0.30 ng/g lipid (range: 0.093–8.1), 0.11 ng/g lipid (range < 0.055–5.8) and 0.18 ng/g lipid (range: < 0.083–45), respectively. In the matched toddler serum samples HBCDDs could not be detected. The authors speculated that the absence of HBCDDs in the serum but high detection frequency in the faeces of the toddlers could be an indication of low uptake and/or fast excretion following the uptake from the gut (see **Section 3.1.1.2.4**). It is noteworthy to state that the LC-MS/MS chromatograms for HBCDDs of the faeces samples showed a further peak, which according to the authors could be δ-HBCDD.

Malarvannan et al. (2013b) demonstrated that HBCDDs can be detected in human hair when they analysed paired human milk and scalp hair samples collected in 2008 from 30 women from the Philippines. While the median for ΣHBCDDs in human milk was 0.19 ng/g lipid (range: < 0.01–0.91), the corresponding median in scalp hair was 0.93 ng/g hair (range: 0.30–5.4). α-HBCDD was the major contributor in both matrices. The authors did not find any association between the levels of HBCDDs measured in human milk and hair samples.

Barghi et al. (2018) analysed human scalp hair for HBCDDs in samples from South Korea and Iran. The concentrations for ΣHBCDDs in the collected hair samples ranged from < LOD (0.04–0.09 ng/g) to 3.24 ng/g with a mean of 0.46 ng/g hair. As HBCDDs could not be determined in hair samples from Iran, but were present in 13 out of 24 samples in hair samples from South Korea, the results indicate a higher exposure to these compounds in South Korea. Based on their experiments, the authors concluded that the HBCDD concentrations in the collected hair samples were mostly coming from endogenous rather than external exposure, and thus, hair can potentially be used as a suitable indicator for monitoring internal exposure to HBCDDs. Martin et al. (2016) could not detect HBCDDs in human hair from six Spanish individuals by LC-MS/MS at an LOD of 11 ng/g hair and an LOQ of 36 ng/g hair.

In summary, the CONTAM Panel concluded that HBCDDs are found in various human tissues and various concentrations, but reliable correlations and associations between different matrices, which are important for biomonitoring purposes, are still lacking.

3.1.1.5. Physiologically Based Kinetic (PBK) modelling

Moreau and Ngong (2019) developed a PBK model in rodents and humans. The model includes six compartments: blood lipoproteins, liver, deep hepatic compartment, adipose tissue, rapidly perfused tissues and slowly perfused tissues. The model was calibrated with the female C57BL/6 mice data from Szabo et al. (2011a,b), a toxicokinetic study where mice were orally administered a single dose of 3 mg/kg of α-HBCDD (see **Section 3.1.1.1**).

According to the authors, the same study was used for the validation of the model, by using the concentrations found in the liver and the blood. To adapt the mouse PBPK model to humans, the

model was scaled to human physiology. The authors performed simulations in human based on the estimations of exposure levels found in the literature. The predicted plasma concentrations were then compared to biomonitoring data from different populations.

According to the authors, the model is '*not ideal for risk assessment for several reasons*'. The same study was used both to calibrate and validate the model. For validation, it is necessary to perform simulations with other independent studies. The validation of the human model was based on estimations of exposure and does not necessarily correspond to the same population where blood concentrations were measured.

The CONTAM Panel considers that the model is not valuable for human risk assessment, e.g. the model was not calibrated in humans (lack of partition coefficients between blood and tissues), and not validated.

3.1.2. Toxicity in experimental animals

EFSA CONTAM Panel (2011a) concluded that main targets in subchronic and chronic toxicity studies in rats and mice were the liver, thyroid and the nervous, reproductive and immune systems. This section provides an overview of the toxicity data described in the previous EFSA Opinion on HBCDDs (EFSA CONTAM Panel, 2011a) with the new data published since, when available.

Several studies were identified in which the animals were exposed to HBCDDs together with other BFRs and/or other POPs (Ernest et al., 2012; Berger et al., 2014; Lefevre et al., 2016; Dianati et al., 2017; Tung et al., 2016, 2017; Allais et al., 2020). The CONTAM Panel did not consider these studies informative for the hazard characterisation of HBCDDs and thus were not considered in this Opinion.

3.1.2.1. Acute toxicity studies

Acute toxicity of HBCDDs is very low. The oral lethal dose in rats is > 20 g/kg bw and > 40 g/kg bw in mice (see EFSA CONTAM Panel, 2011a).

3.1.2.2. Repeated dose toxicity studies

Studies considered in the previous EFSA assessment

In the previous Opinion (EFSA CONTAM Panel, 2011a), several 28 days studies in which HBCDDs were administered to rats described changes in hepatic weight, drug metabolism, gene expression or function. A $BMDL_{20}$ of 22.9 mg/kg bw per day was reported for increased absolute liver weights and a $BMDL_{10}$ of 4.1 mg/kg bw per day was reported for increased glucuronidation of T4 (van der Ven et al., 2006). Increased liver weights were also indicated in adults and in weanling rats in a two-generation dietary study (Ema et al., 2008).

Effects on thyroid were reported in 28-day and 90-day studies, in a two-generation reproductive toxicity study and in a developmental toxicity study. The effects observed were increased hyperplasia and activity of the follicular epithelial cells, increased thyroid weight and follicular cell hypertrophy (Zeller and Kirsch, 1969; Chengelis, 2001; van der Ven et al., 2006; ECB, 2008; ECB, 2008; Ema et al., 2008; Saegusa et al., 2009). The most sensitive effect was increased relative thyroid weight in female rats in the 28-day study with a $BMDL_{10}$ value of 1.6 mg/kg bw per day[29] (van der Ven et al., 2006). However, the CONTAM Panel at that time noted that the dose-response was not clear (at doses up to 30 mg/kg bw, except at the dose 1 mg/kg bw the increase was less than 6%) (EFSA CONTAM Panel, 2011a). The increased relative thyroid weight was accompanied by induction of phase I and phase II biotransformation enzymes in the same animals (LOEL 3 and 30 mg/kg bw per day in females and males, respectively) and T4 glucuronidation (Germer et al., 2006; van der Ven et al., 2006). Endocrine-related effects were reported in several 28-, 90-day and reproductive toxicity studies, such as changes in thyroid hormone homeostasis (decreased serum T4 level and increased TSH level), inhibition of oogenesis, increased anogenital distance, delayed vaginal opening, decreased number of ovarian primordial follicles and decreased the fertility index in rats (Zeller and Kirsch, 1969; Chengelis, 2001; van der Ven et al., 2006, 2009; ECB, 2008; ECB, 2008; Ema et al., 2008; Saegusa et al., 2009; EFSA CONTAM Panel, 2011a). The NOAEL for reduced fertility index and reduction of the number of ovarian primordial follicles was 10 mg/kg bw per day (2-generation reproduction toxicity study in rats) and the $BMDL_5$ for decrease in testes weight was 11.5 mg/kg bw per day (two-generation reproduction toxicity study in rats). The CONTAM Panel concluded in its previous Opinion that activation of constitutive androstane receptor (CAR)- or pregnane-X-receptor (PXR)-dependent gene expression was the likely

[29] BMD values as calculated and reported by the authors of the study.

reason for changes in thyroid hormone homeostasis, and possibly also effects on reproduction, and was considered to be associated with neurodevelopmental effects on behaviour (EFSA CONTAM Panel, 2011a).

Studies published since the previous EFSA assessment

The short-term studies published since the previous EFSA assessment confirms that HBCDDs affect the liver and the thyroid. In addition, HBCDDs affects sex hormones and lipid and sugar metabolism (see **Table 9**).

Several studies in rats and mice reported increased liver weight at doses ≥20 mg/kg bw per day and lesions at doses of 49.5 μg/kg bw per day (Maranghi et al., 2013; Bernhard et al., 2016; Rasinger et al., 2018; Gannon et al., 2019a).

In a 28-day dietary study described by Maranghi et al. (2013) and Rasinger et al. (2018), juvenile female mice were administered HBCDDs (stereoisomers composition not specified) in the diet at doses of 0, 49.5 μg/kg bw per day and 199 mg/kg bw per day. Effects in the liver were increased liver weight at 199 mg/kg bw per day, and lesions (increased vacuolation in hepatocytes, increased pyknotic nuclei, lymphocytic infiltration and hyperaemic vessels) at both doses. However, no difference was observed in the incidence of the lesions between the high dose (199 mg/kg bw per day) and the low dose (49.5 μg/kg bw per day) (see Table D.1 in **Appendix D**). These lesions were not considered further due to the absence of a dose–response relationship. The CONTAM Panel noted that only two doses were tested, there was a wide range between these two doses (4,000-fold apart), the effects observed at low and high doses were reported in two different publications, and the effects on certain organs (uterus, adrenals and brain) were investigated only at the low dose and not at the high dose, precluding the examination of a dose–response relationship for all the effects. Therefore, the CONTAM Panel did not use this study for the establishment of a Reference Point.

Bernhard et al. (2016) exposed juvenile female mice for 28 days to diets spiked with α-HBCDD produced from γ-HBCDD by thermal rearrangement. The doses were 107 μg/kg bw per day and 116 mg/kg bw per day. The control diet contained 46 ng/kg bw per day. In addition, the influence of long-chain omega-3 polyunsaturated fatty acids (LC n3 PUFAs, 4.9 g/kg) on effects induced by α-HBCDD was examined. Effects on liver lipid composition and increased body and liver weights were observed at 116 mg/kg bw per day. There was a marked microvesicular accumulation of lipids at the high dose. These effects were aggravated by LC n3 PUFA intake. Reduction in serum cholesterol occurred at 107 μg/kg bw per day and reduction of serum triacylglycerol were noted at 116 mg/kg bw per day. At the highest dose, an increase of serum aspartate aminotransferase (AST) was observed. There was also a decrease in relative total white adipose tissue weight at high dose. Proteomics of the high-dose group indicated effects on β-oxidation in the liver. The LOAEL was 116 mg/kg bw per day. The CONTAM Panel noted that two doses were tested, but there was a 1,000-fold range between the doses.

In a 28-day study, female and male F344 rats were fed diets containing HBCDDs at 0, 221, 1,250 and 5,000 mg/kg diet (corresponding to 0, 20, 102 and 430 mg/kg bw per day, and to 0, 19, 94 and 400 mg/kg bw per day, for male and female F344 rats, respectively) (Gannon et al., 2019a). Increased relative liver weight was reported in females at the two highest doses. No liver lesions were observed. Increased cholesterol and triglyceride serum levels were observed in female rats at the two highest doses (Gannon et al., 2019a). The authors had initially compared several strains of rats, i.e. Sprague-Dawley, Wistar and Fischer F344, to examine strain- and sex-related differences in response to exposure to HBCDDs, and concluded that the F344 strain was the most sensitive to the effects of HBCDDs on liver and thyroid based on the greatest number of significantly affected endpoints resulting in multiple health effects. Also that in this strain, sex differences were apparent, especially in tissue concentrations, number and severity of thyroid and liver lesions and in immune response parameters, with treatment eliciting a greater response in males (Gannon et al., 2019a).

In a recent study, male Sprague Dawley rats were exposed by gavage to HBCDDs at 0, 0.06, 0.641, 6.41, 64.1 and 641 mg/kg bw per day for 5 days. Increased liver weight (1.2 times compared to control) and incidence of centrilobular hepatocyte hypertrophy was observed at 641 mg/kg bw per day. A significant increase in UDP GT1a1 enzyme was also reported at the two highest doses (Shockley et al., 2020).

Increased thyroid weight, follicular hypertrophy, hyperplasia and/or colloid depletion and vacuolation were reported in rats after 28 days dietary exposure to HBCDDs (Gannon et al., 2019a). Increased thyroid weight was observed in males at the lowest dose of about 20 mg/kg bw per day. Desquamation into follicular lumen, foaming colloid (not statistically significantly increased) and increased ratio of follicular epithelium areas and number of nuclei were observed in a 28-day study in

mice at 49.5 μg/kg bw per day, but not at the high dose of 199 mg/kg bw per day (Maranghi et al., 2013; Rasinger et al., 2014, 2018). Therefore, these effects were not considered further.

In the 28-day study in female mice reported by Maranghi et al. (2013) and Rasinger et al. (2014, 2018), there were statistically significant increased serum testosterone (at 199 mg/kg bw per day) and decreased serum 17β-oestradiol (at 49.5 μg/kg bw per day) concentrations, and increased ratio testosterone to 17β-oestradiol (both doses). The relevance of the hormonal changes observed at 49.5 μg/kg bw per day is unclear as no apical endpoints have been investigated at this dose. Reduction in the number of growing ovarian follicles was also observed in rats at doses ≥ 20 mg/kg bw per day (Gannon et al., 2019a). The CONTAM Panel noted that only female mice were tested.

Exposure of male C57BL/6 mice to HBCDDs at a single dose of 0.05 mg/kg bw per week (equivalent to 0.007 mg/kg bw per day) for 31 weeks was associated with hypertrophy and increased relative weight of epididymal white adipose tissue, with increased lipid accumulation (Xie et al., 2019). The authors provided compelling evidence that this effect was due to an increased expression of *Pparg* mRNA (see **Section 3.1.4**). An increased expression of *Pparg* was also observed in liver of male C57BL/6J mice orally exposed for 14 weeks to an HBCDD dose of 0.035 mg/kg bw per week (equivalent to 5 μg/kg bw per day) in combination with a high-fat diet (containing 330 g lard/kg diet; 506 kcal/kg), which might explain the observed increased accumulation of liver triglycerides and steatosis (Yanagisawa et al., 2014). The combined treatment of HBCDDs and high-fat diet resulted in increased absolute liver weight and body weight gain compared to controls on normal fat diet (AIN-93M; 360 kcal/kg) and to high-fat diet controls (Yanagisawa et al., 2014). The CONTAM Panel noted that both studies administered only one dose level, the relevance to human health of the effects reported by Xie et al. (2019) is unclear and for Yanagisawa et al. (2014) the effects were observed in combination with high-fat diet.

In summary, HBCDDs affect the liver and lipid and sugar metabolism, and induce endocrine-related effects (thyroid and sex hormone homoeostasis).

3.1.2.3. Developmental and reproductive toxicity studies

Studies considered in the previous EFSA assessment

Two dietary reproductive toxicity studies in rats were reported in the previous EFSA assessment (EFSA CONTAM Panel, 2011a). A NOAEL of 10 mg/kg bw per day was established in the 2-generation toxicity study based on a reduced fertility index in F0 animals and a reduction of the number of ovarian primordial follicles in F1 females at 101 mg/kg bw per day (Ema et al., 2008). In the one-generation study, the most sensitive effect on reproductive organs was a decrease in testes weight ($BMDL_5$: 11.5 mg/kg bw per day[28]) (van der Ven et al., 2009). An increased anogenital distance was observed in male pups on PND4 ($BMDL_{10}$: 95.6 mg/kg bw per day[28]) and delayed vaginal opening ($BMDL_{10}$: 82.2 mg/kg bw per day[28]) was observed in female pups. The most sensitive effect observed in this one-generation study was a decrease of the tibia trabecular bone mineral density in F1 females with a $BMDL_{10}$ of 0.056 mg/kg bw per day[28] (van der Ven et al., 2009). The CONTAM Panel noted at that time the ratio between the $BMDL_{10}$ and the $BMDU_{10}$ for this effect was very large (about a factor of 20) indicating a large variation in the dose–response data (EFSA CONTAM Panel, 2011a). A decrease in epididymal weight was also reported in a 28-day dietary study in rats (Zeller and Kirsch, 1969; ECB, 2008).

In developmental toxicity studies in rats, no fetotoxicity, embryotoxicity or teratogenic effects were reported (Murai et al., 1985; Stump, 1999; EFSA CONTAM Panel, 2011a). However, an increased pup mortality during lactation was noted in the F2 generation at 1,008 mg/kg bw per day in the 2-generation reproductive toxicity (Ema et al., 2008).

Studies published since the previous EFSA assessment

No developmental and reproductive studies have been identified since.

3.1.2.4. Immunotoxicity studies

Studies considered in the previous EFSA assessment

In the previous EFSA assessment, effects on the immune system were reported in two studies in rats (EFSA CONTAM Panel, 2011a). In a 28-day study, reduced splenocyte counts were found ($BMDL_{20}$ of 104 mg/kg bw per day[28]) (van der Ven et al., 2006). In a one-generation reproduction study an increased IgG response was found in male offspring after immunisation with sheep red blood cells

(SRBC) ($BMDL_{20}$: 0.46 mg/kg bw per day[28]) as well as an increase in the fraction of neutrophilic granulocytes ($BMDL_{20}$: 7.7 mg/kg bw per day[28]) (van der Ven et al., 2009).

Studies published since the previous EFSA assessment

In the rodent studies published since the previous EFSA assessment (Rasinger, 2011; Maranghi et al., 2013; Rasinger et al., 2014, 2018; Gannon et al., 2019a) (see **Table 9**), the effects on the immune system were confirmed: decreased thymus weight and increased lesions in the thymus (cortical invasivity or signs of tissue stress), decreased splenocyte proliferation and increased spleen lesions, decreased T-cell and increased B-cell population, increased natural killer (NK) cells, decreased serum IgA or IgG immunoglobulin. These effects were observed at doses around 20 mg/kg bw per day in F344 rats.

Table 9: Summary of the outcomes of the toxicological studies of HBCDDs in experimental animals on repeated dose

Test compound	Species	Exposure	Outcome	NO(A)EL/LO(A)EL	Reference
HBCDDs Isomer mixture 'neat' (CAS 3194-55-6) (Promochem) Purity: 99.2% No information on stereoisomer composition	Mice (BALB/c) Juvenile (25 days old at start of the experiment) F	Dietary (fish based), 28 days 10 animals/group Feed ration: 15% (w/w)/kg bw per day 0, 49.5 μg/kg bw per day, 199 mg/kg bw per day Vehicle: DMSO, 0.4 mL/kg feed Control: diet based on casein	*Liver*: Increased relative liver weight (high dose). Increased vacuolation in hepatocytes (at both doses), increased pyknotic nuclei (at both doses), lymphocytic infiltration (at both doses) and hyperaemic vessels (at both doses). *Uterus*: Increase in incidence of reduction in endometrial glands density and irregular multistratification of the luminal epithelium (low dose). *Thymus:* Cortical invasivity (high dose) or signs of tissue stress (Hassal's bodies) (low dose). *Thyroid*: Increased ratio between follicle and colloid areas (at both doses), desquamation into follicular lumen and foaming colloid (low dose), increased ratio of follicular epithelium areas and number of nuclei (low dose). *Spleen*: Increase lymphocyte hyperplasia (high dose). *Hormones:* Lower serum concentration of 17β-oestradiol (low dose); increased concentration of testosterone (high dose); and increase ratio testosterone to 17β-oestradiol (at both doses). See further details in **Appendix C**.	LOEL = 49.5 μg/kg bw per day	Maranghi et al. (2013), Rasinger (2011), Rasinger et al. (2014, 2018). Data from the same animal trial published in three articles and PhD thesis.
HBCDDs Purity: not reported (Sigma-Aldrich) No information on stereoisomer composition	Mice (C57BL/6J) M 5–6 animals/ group	0, 1.75, 35, 700 μg/ kg bw, once per week by gavage Normal and high-fat diet From 6 to 20 weeks	*Metabolic effects*: Body and liver weights increases at the two highest doses, along with paralleled increases in blood glucose and insulin levels and microvesicular steatosis and macrophage accumulation in adipose tissue in the high-dose group.	Increased body and liver weight in combination with high-fat diet: NOEL = 1.75 μg/kg bw/week (or 0.25 μg/kg bw per day) LOEL = 35 μg/kg bw/week (or 5 μg/kg bw per day)	Yanagisawa et al. (2014)
α-HBCDD Produced from γ-HBCDD by thermal rearrangement Purity: not reported	Mice (BALB/c) F 3 weeks of age 8 animals/group	Dietary, 28 days 46 ng/kg bw (control), 107 μg/kg bw per day, 116 mg/kg bw per day LC n-3 PUFAs: 4.9 g/kg	*Influence of LC n-3 PUFAs on α-HBCDD mediated effects*: The highest dose of α-HBCDD affected liver lipid composition and increased body and liver weight as aggravated by high PUFA intake. The highest dose also reduced thymus weight. The lowest dose slightly reduced body weight gain and liver somatic index. The lowest dose reduced serum cholesterol and the highest dose reduced triacylglycerol. Increased AST serum level was observed at the highest dose and an increase in catalase activity was noted at the low dose. Proteomics of the high-dose group indicated effects on ß-oxidation in liver.	Some effects at the lowest dose (107 μg/kg bw per day) but different directionality compared with the highest dose (116 mg/kg bw per day). LOAEL= 116 mg/kg bw per day	Bernhard et al. (2016)

Test compound	Species	Exposure	Outcome	NO(A)EL/LO(A)EL	Reference
HBCDDs Purity: 95% (Sigma Aldrich) 1% α-, 1% β-, 98% γ-HBCDD	Rats (F344 M and F, Sprague-Dawley F, Wistar F) 34–37 days old	Dietary, 28 days 0, 250, 1,250, 5,000 mg/kg diet (measured levels in the diet: 0, 221, 1,125, 3,549 mg/kg diet) Calculated daily consumption: F F344 rats: 0, 20, 102, 430 mg/kg bw per day M F334 rats: 0, 19, 94, 400 mg/kg bw per day F Sprague-Dawley rats: 0, 21, 107, 412 mg/kg bw per day F Wistar rats: 0, 20, 112, 466 mg/kg bw per day 5 animals/group	*Effects on liver, thyroid gland, immune system and endocrine system*: Sex differences in metabolism (tissue concentration levels), immune response parameters and in number and severity of thyroid and liver lesions following exposure to T-HBCDD, with greater responses in M. Wistar rats: thyroid (minimal to mild follicular hypertrophy). Sprague Dawley rats: liver (increase relative weight), thyroid (mild follicular hypertrophy). Decreased T-cell and increased B-cell population. F344 rats: liver (increase relative weight in F); thyroid (increase relative weight in M, mild follicular hypertrophy and colloid depletion); brain (decrease relative weight in F); kidney (mild chronic nephropathy and mild multifocal tubular mineralisation in all treated M); spleen (atrophy in two high-dose M, decrease splenocytes proliferation in M and F); ovaries: (significant reduction in the number of growing follicles). Decrease in IgG serum immunoglobulin in F. Decreased T-cell and increased B-cell population, increase in NK-cells in M. Changes in multiple haematological parameters in M and F at the two highest doses. Changes in clinical chemistry parameters (AST and creatine kinase) in all strains, more pronounced in F344 rats. Decrease serum T4 level at the three dose levels in M F344 rats. Increase cholesterol and triglycerides in the two high doses in F F344 rats. Wistar and Sprague Dawley rats: Concentration of γ-isomer was more abundant than α-isomer in serum, adipose tissues and also in the liver (lower concentration); the β-isomer was not detected. F344 rats: higher α- and γ- isomers accumulation in F compared to M, except for the α-isomer in serum.	LOAEL F344 rats = 19 mg/kg bw per day LOAEL F Wistar rats = 20 mg/kg bw per day LOAEL F Sprague-Dawley rats = 21 mg/kg bw per day	Gannon et al. (2019a)

Test compound	Species	Exposure	Outcome	NO(A)EL/LO(A)EL	Reference
HBCDDs enriched with α-HBCDD (A-HBCDD) Purity: 95% (Technical HBCDD) (Sigma Aldrich) 81% α-, 7% β-, 12% γ-HBCDD	Rats (F344) F, M 5 animals/group	Dietary, 28 days 0, 250, 1,250, 5,000 mg/kg diet (measured levels in the diet: 0, 200, 898, 3,938 mg/kg diet) Calculated daily consumption: M: 0, 19, 97, 395 mg/kg bw per day F: 0, 19, 99, 363 mg/kg bw per day	*Effects on liver, thyroid gland, immune system and endocrine system*: Increased relative liver weight at two high doses in M and F, and thyroid weight at high dose in M and F, decreased thymus and brain weight in two high-dose F. Changes in haematological and clinical chemistry parameters in M and F. A-HBCDD residue concentrations: α-isomer was more abundant than the γ-isomer in serum, adipose tissues and also in the liver (lower concentration). Significantly higher A-HBCDD accumulation in F. Histopathological effects: liver (increased incidence and severity zone 3 hepatocellular hypertrophy), thyroid (dose-related increase in incidence and severity of follicular hypertrophy and hyperplasia, colloid depletion), spleen (dose-related increase in lesions: mild to marked PALS atrophy, mild follicular and MZ atrophy), ovaries (increase proportion of growing follicles) Decrease in splenocytes proliferation. Increase in serum immunoglobulin IgA at high dose. Changes in blood lymphocytes populations: decrease in T_H cells and increase in T_c cells in high-dose F, but no change in T-cell/B-cell ratio; changes in T-cell and B-cell populations at the highest dose in M, decrease in TH cells, T_H/T_c ratio and increase in NK and B cells. Treatment-related effect in thymus lymphocyte subpopulations in M.	NOAEL = 19 mg/kg bw per day	Gannon et al. (2019a)
HBCDDs Purity: not reported (Sigma-Aldrich) No information on stereoisomer composition	Mice (C57BL/6) M 3–4 weeks of age 6 animals/group	0, 50 μg/kg bw per week For 31 weeks from 3–4 weeks of age	Adipogenic effects. Increased epididymal white adipose tissue weight and hypertrophy.	Increased epididymal white adipose tissue weight and hypertrophy: LOEL = 50 μg/kg bw per week	Xie et al. (2019)

Test compound	Species	Exposure	Outcome	NO(A)EL/LO(A)EL	Reference
HBCDDs Purity: 96% (Gojira Fine Chemicals) No information on stereoisomer composition	Rat (Harlan Sprague Dawley) M 7-week old 6 animals/group	0, 0.1, 1, 10, 100, 1,000 μM/kg bw per day (corresponding to 0, 0.06, 0.641, 6.41, 64.1, 641 mg/kg bw per day, as reported by the authors) Gavage 5 days Vehicle: corn oil	Liver: increased weight (1.2 X) and incidence of centrilobular hepatocyte hypertrophy at HD and significant increase UDP GT1a1 enzyme at two highest doses. Thyroid: no histological changes.	NOAEL = 6.41 mg/kg bw per day	Shockley et al. (2020)

AST: aspartate aminotransferase; BMDL: benchmark dose lowest confidence interval; DMSO: Dimethyl sulfoxide. F: female; HBCDDs: hexabromocyclododecanes; IgA: immunoglobulin A; IgG: Immunoglobulin G; M: male; LC n-3 PUFAs: long-chain omega-3 polyunsaturated fatty acids; MZ atrophy: marginal zone atrophy; LOEL: lowest-observed effect; LOAEL: lowest-adverse-effects level; NK-cells: natural killer cells; NOEL: no-observed-effect level; PALS: periarteriolar lymphatic sheaths; T_c cells: cytotoxic T cells; T_H cells: T helper cells; T4: thyroxine.

3.1.2.5. Neurotoxicity studies

Studies considered in the previous EFSA assessment

In the previous Opinion, the CONTAM Panel concluded that exposure of rodents to HBCDDs during development affects the nervous system with subsequent behavioural changes (EFSA CONTAM Panel, 2011a).

Neurodevelopmental effects were described in F1 and F2 offspring of rats receiving 15,000 mg/kg diet HBCDDs (Ema et al., 2008). In a one-generation study in rats, hearing alterations occurred in male rats and changes in haloperidol-induced catalepsy mainly in females ($BMDL_5$ were 0.2 and 0.9 mg/kg bw per day[28] for increased thresholds in the brainstem auditory evoked potentials (BAEP) and 0.6–4.4 mg/kg bw per day[28] for reduced latencies to movement onset in catalepsy) (Lilienthal et al., 2009).

The critical study selected by the CONTAM Panel at that time involved a single oral administration of HBCDDs (98% purity, proportions of α-, β- and γ-HBCDD were 3%, 8% and 89%, respectively) via gavage to 10-day old male and female mice at doses of 0, 0.9 or 13.5 mg/kg bw (Eriksson et al., 2006). When the mice were three months old, spontaneous behaviour, spatial learning and memory were assessed and dose-related changes reported. Only male mice were examined. Ten mice per group (from 3–4 L) were tested for spontaneous behaviour (horizontal locomotion, rearing and total activity) for three 20-min intervals of a 60-min observation period. The HBCDD-exposed mice showed dose-dependent hypoactivity in the first 20 min, while they were more active at the end of the observation period. Groups of 12–17 mice (from 3–4 L) were assessed for spatial learning and memory in a Morris swim maze. The mean latencies were longer in mice dosed HBCDDs at 13.5 mg/kg bw but not at 0.9 mg/kg bw, compared to controls. The NOAEL for spatial learning and memory was 0.9 mg/kg bw. Based on the findings for horizontal locomotion and rearing, the LOAEL in this study was 0.9 mg/kg bw HBCDDs, the lowest dose tested. The Panel performed dose–response modelling on the results for horizontal locomotion, rearing and total activity in the first 20-min period. The CONTAM Panel at that time selected horizontal locomotion and total activity as more reliable parameters than rearing for the analysis. Of these, the lowest $BMDL_{10}$ was obtained for horizontal locomotion and this was used by the then CONTAM Panel as the Reference Point in its previous risk assessment (EFSA CONTAM Panel, 2011a).

Neurobehavioural effects were also reported in reproductive and developmental dietary studies with rats that were reviewed in the previous EFSA assessment (see **Section 3.1.2.3**), but at higher doses than the effects described above. In these studies (Ema et al., 2008; van der Ven et al., 2009), the endpoints measured, and age of observation, differed from those in the study of Eriksson et al. (2006).

Studies published since the previous EFSA assessment

Most of the new studies published since the previous EFSA assessment were based on repeated exposures to HBCDDs performed by exposing dams or neonatal rodents at different developmental stages, i.e. GD0-PND21 (Maurice et al., 2015), GD1-PND0 (Miller-Rhodes et al., 2014) and PND10-PND70 (Zhang et al., 2017a), followed by behavioural analyses of the offspring at different ages. One study exposed adult animals for 6 weeks (Pham-Lake et al., 2017) (see **Table 10**).

Only one study provided information on a specific stereoisomer, i.e. alpha-HBCDD (Maurice et al., 2015). The other studies were conducted with HBCDDs for which the stereoisomer composition was not reported (Miller-Rhodes et al., 2014; Zhang et al., 2017a).

In Miller-Rhodes et al. (2014), offspring of rats given HBCDDs by gavage from GD1 to PND0 at doses of 0, 3, 10 or 30 mg/kg bw per day, showed significant neurodevelopmental effects at all doses, but with no clear dose–response relationships. These effects consisted of increased reactivity to a tail pinch in neonates, decreased forelimb grip strength in juveniles, impaired sustained attention and loss of hind leg function, which progressed and increased in severity with age up to 21 months (Miller-Rhodes et al., 2014).

Maurice et al. (2015) found impaired motor activity and reduced anxiety, associated with decreased pup weight, at 22 ng/kg bw per day in offspring of female rats dosed with hens' egg contaminated with α-HBCDD by gavage from GD0 to PND21. No effects were reported at a three-fold higher dose of 66 ng/kg bw per day.

In Zhang et al. (2017a), 10-day old Sprague-Dawley rats (sex not specified, 9 animals/group) were exposed by gavage to 0, 0.3, 3 and 30 mg/kg bw HBCDDs for 60 consecutive days (from PND10 to PND70). Using the Morris water maze test, the authors reported a dose-related impaired spatial

learning and memory ability estimated by the increase in the latency time of the animals seeking the platform ($p < 0.05$) at 3 and 30 mg/kg bw at all time points (day 3, 4 and 5). This trend was also present for the 0.3 mg/kg bw group, but was significant on day 5, only. In addition, they observed a decrease in the time remaining in the target quadrant where the platform had been placed and in the number of times that each rat had crossed the non-exits. Downregulation of neurotropic factors in the hippocampus in rats dosed with HBCDDs at 3 or 30 mg/kg bw per day HBCDDs was also observed. The CONTAM Panel noted the lack of information on the protocol and the poor reporting of the results. For example, the number of litters, the sex of the animals and the age at observation were not stated. For the last, the Panel assumed that this was at the end of the 60-days dosing period. The Panel also noted that the number of animals tested is low, and that the data were very poorly displayed for the Morris water maze (the presentation of the statistics is poor). Therefore, it is difficult to assess whether the increase in latency is robust or due to chance. Locomotor activity (swim speed) and anxiety were not assessed, and they can be key confounders of other behaviours. General health condition of the animals was not reported, e.g. body weight. Learning curves were displayed for only days 3–5. Expression of neurotropic factors (brain-derived neurotropic factor (BDNF), nerve growth factor (NGF) and fibroblast growth factor (FGF)) was investigated. However, the antibodies used for the analysis did not allow for distinction between the different FGF family members with different function expressed in the brain. Changes in the expression of these factors were observed at different doses, but no causality can be established. It is also noted that these data were derived from adult rats (> 75 days old) and thus interpretation related to early development are difficult to make. Moreover, the litter effect was not investigated. The Panel concluded that the study shows an effect on neurodevelopment in rats. However, due to the limitations described above, this study is not considered further for the derivation of a Reference Point.

Pham-Lake et al. (2017) reported that 2-month old male mice dosed with 25 mg/kg bw per day HBCDDs by gavage for 6 weeks, followed by a further 6 weeks without dosing, did not exhibit explicit behavioural abnormalities such as deficits in grooming, isolation, feeding or generalised movement. The absence of these effects does not necessarily contradict those observed by Eriksson et al. (2006) and Zhang et al. (2017a) at an earlier stage of development. They were induced at a different time window and the endpoints evaluated are different.

None of the other new animal neurotoxicity studies with HBCDDs specifically assessed neurobehavioural endpoints but investigated molecular and cellular effects in the brain (Saegusa et al., 2012; Rasinger et al., 2014, 2018; Genskow et al., 2015; Pham-Lake et al., 2017; Reffatto et al., 2018) (see Sections 3.1.4 and 3.1.5).

The CONTAM Panel re-assessed the study of Eriksson et al. (2006), which had been selected as the critical study in the previous EFSA assessment, and noted several limitations, such as the small number of animals tested (males only) from three to four litters, investigation of the litter effect was not reported and the fact that HBCDDs were administered on only one day (PND10). Furthermore, there was a limited range of tests conducted. The mice were tested as adults and key adaptations across adolescence could have altered their behaviour as adults. Anxiety, exploration, motivation and motor ability were not assessed; changes in these parameters could have explained the changes in spontaneous motor behaviour. However, the CONTAM Panel noted that PND10 does represent the start of a critical period in the development of the brain in the rodents under study and the observations on spontaneous behaviour (horizontal locomotion, rearing and total activity) could not be discounted. In the Morris water maze, there were a limited number of trials, a short trial duration and only latency to reach the platform was measured. In addition, swim speed and anxiety were not assessed. Due to these limitations in the assessment of spatial learning and memory, the CONTAM Panel did not consider this endpoint for the derivation of a Reference Point. Acknowledging the limitations in the study, the Panel identified a LOAEL of 0.9 mg/kg bw based on the decrease in spontaneous behaviour (horizontal locomotion, rearing and total activity).

In summary, neurodevelopmental effects were described in offspring of rats given HBCDDs by gavage from GD1 to PND0 at doses from 3 to 30 mg/kg bw per day (Miller-Rhodes et al. (2014). A dose-dependent decrease in spontaneous behaviour (horizontal locomotion, rearing and total activity) was noted with a LOAEL of 0.9 mg HBCDDs/kg bw (Eriksson et al., 2006). In addition, impaired spatial learning and memory were observed in mice exposed by gavage to 13.5 mg/kg bw HBCDDs on PND10 (Eriksson et al., 2006) as well as in rats exposed by gavage to 0.3–30 mg/kg bw per day from PND10 to PND70 (Zhang et al., 2017a). Due to the limitations identified (see above), spatial learning and memory was not considered for the derivation of a Reference Point.

Table 10: Summary of the behavioural studies of HBCDDs in experimental animals

Test compound	Species	Exposure	Outcome	NO(A)EL/ LO(A)EL	Reference
HBCDDs (3% α-, 8% β- and 89% γ-HBCDD, as reported in Fång, 2007) Purity: > 98% (prepared from a commercial mixture by the research group of Professor Bergman at the Department of Environmental Chemistry, Stockholm University)	Mice (NMRI) M Neonatal pups	Gavage, at PND10 0, 0.9, 13.5 mg/kg bw (single dose) Vehicle: fat emulsion	Spontaneous behaviour, spatial learning and memory were assessed at age 3 months. At 0.9 and 13.5 mg/kg bw, decreased horizontal locomotion and rearing. Impaired spatial learning and memory (as observed in a Morris water maze) at 13.5 mg/kg bw but not 0.9 mg/kg bw.	LOAEL for spontaneous behaviour = 0.9 mg/kg bw	Eriksson et al. (2006)
HBCDDs Purity: ≥ 95% (Sigma-Aldrich) No information on stereoisomer composition	Rats (Long-Evans) M, F pregnant 8–11 animals/group	Gavage, from GD1 to PND0 0, 3, 10, 30 mg/kg bw per day Vehicle: corn oil Litters normalised to 8.	Multiple neurobehavioural tests performed on offspring at different ages up to 21 months. HBCDDs produced age-related effects at 3 mg/kg bw per day (increased reactivity to a tail pinch in neonates, decreased forelimb grip strength in juveniles, impaired sustained attention and progressive loss of hind leg function in aged rats). No effect on locomotor activity.	No clear dose–response relationships.	Miller-Rhodes et al. (2014)
α-HBCDD Purity: 99.3% (produced by transformation of γ-HBCDD by heat treatment, followed by purification)	Rats (Wistar) F n = 6	Gavage of hen's egg produced by feeding hens with α-HBCDD, from GD0 to PND21 0, 22, 66 ng/kg bw/day Not stated whether litters were normalised	Decreased pup weight at 22 ng/kg bw per day, associated with impaired motor activity and decreased anxiety. No effects at 66 ng/kg bw per day.	No clear dose–response relationships	Maurice et al. (2015)
HBCDDs Purity: not reported (Sigma) No information on stereoisomer composition	Rats (Sprague Dawley)(b) Sex not specified 10 days of age 9 animals/group	Gavage, from PND10 to PND70 0.3, 3, 30 mg/kg bw per day 30 mg/kg HBCDD + 300 mg/kg bw per day taurine	Impaired spatial learning and memory in the Morris water maze test. Expression of BDNF, NGF and FGF(c) in the hippocampus increased at low dose (adaptive) and impaired at higher doses. Taurine appeared to be protective when co-administered.	Not identified due to limitations in the study (see study description above)	Zhang et al. (2017a)

Test compound	Species	Exposure	Outcome	NO(A)EL/ LO(A)EL	Reference
HBCDDs Purity: not reported (Sigma-Aldrich) No information on stereoisomer composition	Mice (C57BL/6J) M 4 animals/group (control), 6 animals/group (treatment)	Gavage, 6 weeks 0, 25 mg/kg bw per day From 2 months old Controls: corn oil	Decreased expression of presynaptic, but not post-synaptic, dopaminergic proteins in hypocampus. No overt behavioural abnormalities (such as deficits in grooming, isolation, feeding or generalised movement.	LOEL = 25 mg/kg bw per day	Pham-Lake et al. (2017)

F: female; M: male. BDNF: brain-derived neurotropic factor; NGF: Nerve growth factor; FGF: Fibroblast growth factor; PND: postnatal day; GD: gestational day.
(a): In the absence of factors for converting concentrations in feed into daily doses for pregnant rats, the default factor of 0.12 for subactute studies in rats was used (EFSA Scientific Committee, 2012).
(b): The manuscript sometimes refers to mice, which is assumed to be an error.
(c): Expression of specific FGF forms was not investigated.

3.1.2.6. Genotoxicity studies

Studies considered in the previous EFSA assessment

Based on the negative results from the studies reported in EFSA CONTAM Panel (2011a), i.e. a reverse mutation assay in S. Typhimurium, an *in vitro* chromosomal aberration test in human peripheral blood lymphocyte, and an *in vivo* micronucleus test in mice after i.p. administration, it was concluded in the previous assessment that HBCDDs are not genotoxic *in vitro* and *in vivo*.

Studies published since the previous EFSA assessment

Since then, the following studies have been published.

Li et al. (2017b) exposed the human breast HBL-100 cell line to HBCDDs at 0, 5, 10 and 50 mg/L for 24 h. The cytotoxicity (as measured by the MTT and LDH assays) was significantly reduced at 50 mg/L (reduction of 60%). Oxidative stress (measured by reactive oxygen species (ROS) content) was also induced at 50 mg/L. A statistically significant increase in DNA strand breaks (as measured by % tail DNA, in Comet assays) was observed only at the highest and cytotoxic dose (Li et al., 2017b).

No increase of DNA strand breaks was observed in an alkaline Comet assay where human hepatocyte L02 cells (human fetal hepatocyte cell line) were exposed for 24, 48 or 72 h to low concentrations of HBCDDs (10^{-13} to 10^{-7} M). However, high concentrations of HBCDDs (20, 40 and 60 μmol/L) caused a significant elevation of DNA strand breaks in a time-dependent manner. High concentrations of HBCDDs (> 20 μmol/L) significantly reduced cell numbers in a concentration and time dependent manner, while lower concentrations of HBCDDs for 72 h slightly increased cell survival compared with the control. The ROS level in HBCDD treated cells was elevated in a time-dependent manner. It was also shown that the ROS level induced by low concentrations of HBCDDs was comparatively lower than that induced by high concentrations of HBCDDs (An et al., 2013).

High concentration of HBCDDs (50 μmol/L) caused significant increases in DNA strand breaks in L02 cells (human fetal hepatocyte cell line) as measured by the alkaline Comet assay. Low concentrations of HBCDDs alone have no obvious effects on the cell viability, while 50 μM HBCDDs significantly suppressed cell survival. However, a pretreatment with low concentrations of HBCDDs alleviated the cytotoxicity induced by high concentrations of HBCDDs. When the cells were pretreated with low concentrations of HBCDDs for 48 h, the ROS levels induced by subsequent treatment with 50 μmol/L HBCDDs were markedly lower than those in the cells treated with 50 μmol/L HBCDDs alone. The authors concluded that pretreatment with low concentrations of HBCDDs induced 'adaptive responses' to high concentrations of HBCDDs exposure, as evidenced by attenuation of toxicity, of ROS production and of DNA strand breaks induction (An et al., 2016).

Concentration-related statistically significant increases in Tail DNA % were observed in a Comet assay in human acute monocytic leukaemia THP-1 cells exposed for 1 h to 5–100 μg/mL HBCDDs. No information on cytotoxicity was provided (Wang et al., 2020).

The CONTAM Panel concluded that this information would not change the previous conclusion that HBCDDs are not genotoxic *in vitro* or *in vivo*. The slight induction of DNA strand breaks observed in some *in vitro* tests is most likely due to oxidative stress (see also **Section 3.1.4**).

3.1.2.7. Carcinogenicity

Studies considered in the previous EFSA assessment

Only one carcinogenicity study was available at the time of the previous EFSA assessment (Kurokawa et al., 1984; ECB, 2008; EFSA CONTAM Panel, 2011a). In that study male and female mice were exposed to HBCDDs (information on stereoisomer composition not provided) at 0, 13, 130 or 1,300 mg/kg bw per day in their diet for 18 months. The incidence of liver carcinomas was increased in females, without a dose–response relationship and the incidences of these tumours were within the background ranges in this strain of mouse. The CONTAM Panel concluded at that time that '*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*' (EFSA, 2011a).

Studies published since the previous EFSA assessment

No new carcinogenicity studies have been identified.

3.1.3. Observations in humans

In the previous EFSA Opinion on HBCDDs (EFSA CONTAM Panel, 2011a), two cross-sectional epidemiological studies on bone mineral density and thyroid stimulating hormone were identified showing inconclusive results (Weiss et al., 2006; Eggesbø et al., 2011). Since then, 15 additional epidemiological publications were identified corresponding to 13 study populations assessing the association between exposure to HBCDDs and any endpoint related to human health (see **Table 11**). Opting for the totality of the evidence, this section also discusses the two studies already described in the previous Opinion.

The evidence base includes one cohort study, one birth cohort study (reported in four publications) and 11 cross-sectional studies where the HBCDD exposure was assessed simultaneously or even later than the endpoint ascertainment. The sample size of the included observational studies ranged from 34 to 71,415 participants. All the evaluated populations came from European countries except for five cross-sectional studies in which populations from the USA (n = 2), China, South Korea and Tanzania were investigated.

The populations under study were diverse. Four studies recruited younger children or adolescents, while the remaining studies assessed adult female (n = 6), male (n = 2) or mixed (n = 1) populations. HBCDD exposure was assessed via serum biomarkers (n = 9), biomarkers in breast milk (n = 1), biomarkers in adipose tissue (n = 1), HBCDD measurements in dust (n = 1), or through merging dietary patterns and presence of HBCDDs in food samples (n = 1). Birth weight/length, neurodevelopment and thyroid dysfunction were the three endpoint categories used in children. Subfertility, type 2 diabetes, thyroid hormone levels, severe endometriosis and ovarian endometrioma and breast cancer metastasis were the endpoints assessed in the adult populations.

In the following paragraphs, the available evidence base is reported in detail ordered by study design (cohorts, birth cohorts, cross-sectional studies), method of exposure assessment and year of publication.

Ongono et al. (2019) reported on the only available observational study conducted on the association between HBCDDs and type 2 diabetes (T2D); 71,415 middle-aged women were followed for 19 years in France (3,667 incident T2D cases, 3% attrition). The dietary exposure to HBCDDs was calculated by merging the usual food consumption over the previous year estimated through a validated 208-item semi-quantitative dietary questionnaire sent in 1993 and food contamination data available from the 2nd French Total Diet Study (TDS2) published by Anses in 2011. Exposure to PBDEs was also assessed independently without being included in the analysis for HBCDDs. The dietary exposure to HBCDDs was calculated for quintiles and these ranged from 0.11 to 0.35 ng/kg bw per day with a mean of 0.22 ng/kg bw per day. There was a statistically significant linear association between the dietary exposure per quintile to HBCDDs and T2D (HR: 1.18; 95% CI: 1.06–1.30 for the 2nd quintile up to HR: 1.47; 95% CI: 1.29–1.67 for the 5th quintile group). The model was adjusted for several factors including body mass index (BMI), physical activity, smoking status, level of education. An association with obesity as a binary endpoint was not investigated. However, due to the fact that BFRs are highly lipophilic and accumulate in fats, the interaction between HBCDDs (or PBDEs) and BMI was tested; there were no indications for effect modification of the relation between HBCDDs (ng/kg bw per day) and T2D risk by BMI ($\leq$ 25 and > 25 kg/m^2) ($p_{interaction}$ = 0.9).

The Groningen Infant COMPARE[30] (GIC) birth cohort was founded in 2001 in The Netherlands and consisted of 90 healthy pregnant women, living in the northern provinces of The Netherlands, who delivered a single, full term, healthy infant. HBCDDs were measured through LC/MS-MS in maternal serum at the 35th week of pregnancy and the observed serum levels were relatively low (median, interquartile range (IQR), range, ng/g lipid; 0.8, 0.47–1.26, 0.3–7.5). Seven additional neutral organohalogen compounds were also assessed (4,4′-DDE, PCB-153, BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154) as well as four phenolic organohalogen compounds (4-hydroxy-2,3,3′,4′,5-pentachlorobiphenyl (4OH-CB-107), 4-hydroxy-2,2′,3,4′,5,5′-hexachlorobiphenyl (4OH-CB-146), 4-hydroxy-2,2′,3,4′,5,5′,6-heptachlorobiphenyl (4OH-CB-187) and PCP). Exposure to these compounds was assessed independently without being included in an adjusted analysis. Four publications reported on the association between HBCDDs exposure and health-related endpoints in this cohort. Meijer et al. (2012) addressed the association between prenatal HBCDD exposure and testosterone, free testosterone, sex hormone-binding globulin (SHBG), LH, FSH, estradiol (E2), free E2 (FE2) and inhibin

[30] Comparison of Exposure-Effect Pathways to Improve the Assessment of Human Health Risks of Complex Environmental Mixtures of Organohalogens.

B (InhB), at the age of 3 months, and testes volume and penile length at the age of 3 and 18 months. No statistically significant association was found. Ruel et al. (2019) investigated the association between exposure to organohalogen compounds (including HBCDDs) and child development (mental and motor) at the age of 18 months. No statistically significant association was found. Roze et al. (2009) reported on an extensive follow-up of the study that assessed neuropsychological functioning including motor performance (coordination, fine motor skills), cognition (intelligence, visual perception, visuomotor integration, inhibitory control, verbal memory and attention) and behaviour at 5–6 years of age. Among this large number of assessed endpoints, HBCDD exposure (maternal serum) was statistically significantly positively associated with coordination (rho, 0.29; p value, 0.023), total intelligence (rho, 0.39; p-value, < 0.05) and verbal intelligence (rho, 0.48; p value, < 0.01).Berghuis et al. (2018) in a longer follow-up reported on endpoints related to cognition and motor performance in adolescents. Attempting to extend an earlier finding at the 5–6 years follow-up, HBCDD exposure was marginally statistically significantly and negatively associated with total intelligence (WISC-III-NL[31]; rho −0.355; 95% CI, −5.854, −0.001, p-value 0.05). No statistically significant association was found for performance intelligence, sustained auditory attention, verbal intelligence, auditory-verbal memory, selective visual attention, or motor endpoint.

In addition to the two cohort studies described in detail above, 11 cross-sectional studies (sample size range: 38–515) were identified, each assessing a different association except for thyroid hormone levels which were assessed in three studies. The endpoints investigated include breast cancer metastasis (n = 91, Koual et al., 2019), severe endometriosis (Ploteau et al., 2017; Matta et al., 2020), subfertility (n = 163, Den Hond et al., 2015), congenital hypothyroidism (n = 38, Kim and Oh, 2014), thyroid hormone levels in occupationally exposed adults (n = 80, Li et al., 2014), in pregnant women (n = 140, Stapleton et al., 2011) and in neonates (n = 193, Eggesbø et al., 2011), sex-related hormones (n = 62, Johnson et al., 2013), birth weight and birth length (n = 150, Müller et al., 2016), neurodevelopment in adolescents (n = 515, Kicinski et al., 2012) and bone mineral density (n = 50, Weiss et al., 2006). Of these, statistically significant results were reported for the associations between lower α-HBCDD levels in serum of mothers after delivery and the risk of having a child born with congenital hypothyroidism (Kim and Oh, 2014) and between HBCDD levels in house dust from men recruited through a US infertility clinic and free androgen index and sex hormone binding globulin (Johnson et al., 2013). Of note, the Müller et al. (2016) study attempted to assess the association between HBCDDs in breast milk and birth weight and length in Northern Tanzania. HBCDDs were detected above the LOD in one breast milk samples only and further association analyses were not applicable. Stapleton et al. (2011) also attempted to assess the association between HBCDDs (α-, β- and γ-isomers) and thyroid hormone levels in pregnant women but in none of the samples HBCDDs were detected above the LOD.

In summary, there is a growing body of epidemiological research in the field of adverse events related to HBCDDs exposure. The endpoints assessed pertained to type 2 diabetes and neurodevelopment in the longitudinal studies. In cross-sectional studies the endpoints under consideration (each appraised in a single study) included breast cancer metastasis, severe endometriosis, subfertility, congenital hypothyroidism, thyroid hormone levels in occupationally exposed adults, in pregnant women and in neonates, sex-related hormones, birth weight and birth length, neurodevelopment in adolescents and bone mineral density. None of the effects studied in the longitudinal studies assessing internal exposure either reached statistical significance or were replicated in a longer follow-up point in the same study. The currently available evidence is characterised by relatively small sample sizes in most of the studies, considerable heterogeneity in the assessed populations, exposures and endpoints, varying methodological quality, and effect inconsistency. Moreover, at present no studies were identified with the same endpoint neither in the paediatric nor in the adult populations. Hence, the ability to confirm the postulated associations is limited. Moreover, confounding introduced by potential underlying associations with other contaminants was scarcely addressed analytically.

[31] The Wechsler Intelligence Scale for Children (WISC) is an intelligence test for children between the ages of 6 and 16. Its output is a Full Scale Intelligence Quotient and five index scores (Verbal Comprehension Index, Visual Spatial Index, Fluid Reasoning Index, Working Memory Index, and Processing Speed Index).

Table 11: Summary of epidemiological studies on HBCDDs

Reference	Recruitment year Country	Study design Follow-up (years)	N Population group (age) (years) Gender (% males)	Compound Tissue analysed Method (LOD/LOQ)	Levels of Exposure (ng/g lipid)[a]	Endpoint health category	Effect estimate (95% CI), p-value
Ongono et al. (2019)	1990 France	Cohort 19	71,415 Adults (52.87 ± 6.66) 0%	ΣHBCDDs Food samples LC-MS/MS	0.22 ng/kg bw per day[b]	Diabetes type 2	1.18 (1.06–1.30) NS; 1.28 (1.15–1.42 NS; 1.35 (1.20–1.51) NS; 1.47 (1.29–1.67) NS[c]
Meijer et al. (2012)	2001–2002 The Netherlands	Birth cohort 0.25, 1.5	34 Infants (0.25, 1.5) 100%	ΣHBCDDs Maternal serum LC-MS/MS (NR)	0.7 (ND–7.4)	Free testosterone	–0.31 (NR) NS[d]
Ruel et al. (2019)	2001–2002 The Netherlands	Birth cohort 1.5	59 Children (1.5) 63%	ΣHBCDDs Maternal serum LC-MS/MS (NR)	0.82 (0.47–1.26)	BSID-II-NL	−0.133 (NR) NS[d]
						MDI	0.004 (NR) NS[d]
Roze et al. (2009)	2001–2002 The Netherlands	Birth cohort 5–6	62 Toddlers (5–6) 61%	ΣHBCDDs Maternal serum LC-MS/MS (NR)	0.8 (0.3–7.5)	Coordination	0.29 (NR) NS[d]
						Total intelligence (WISC-III-NL)	0.39 (NR) < 0.05[d]
						Verbal intelligence (WISC-III-NL)	0.48 (NR) < 0.01[d]
						Motor performance and behaviour[e]	NR (NR) NS[d]
Berghuis et al. (2018)	2001–2002 The Netherlands	Birth cohort 13–15	31 Adolescents (13–15) 100%	ΣHBCDDs Maternal serum LC-MS/MS (NR)	0.79 (0.46–1.31)	Performance intelligence (WISC-III-NL)	−0.352[d] (−6.525, 0.029) 0.052[f]
						Total intelligence (WISC-III-NL)	−0.355[d] (−5.854, −0.001) 0.050[f]
						Sustained auditory attention ('Score!', TEA-Ch-NL)	−0.301[d] (−13.739, 0.913) 0.084[f]
						Neurodevelopment[f]	NR (NR) NS[d]
						Reproductive development[g]	NR (NR) NR
Koual et al. (2019)	2013–2017 France	Cross-sectional NA	91 Adults (64.0, 51.0–73.0) 0%	α-HBCDD[h] Adipose tissue LC-MS/MS (NR)	1.62 (0.97–1.95)	Breast cancer metastasis	NR (NR) 0.18

Reference	Recruitment year Country	Study design Follow-up (years)	N Population group (age) (years) Gender (% males)	Compound Tissue analysed Method (LOD/LOQ)	Levels of Exposure (ng/g lipid)[a]	Endpoint health category	Effect estimate (95% CI), p-value
Ploteau et al. (2017), Matta et al. (2020)	2013–2015 France	Cross-sectional NA	99 Adults (18-45) 0%	α-HBCDD Adipose tissue LC-MS/MS (NR)	Controls; 0.70, IQR (0.43–1.39)	Severe endometriosis[i]	NR (NR) NS[j]
			74 Adults (18-45) 0%	α-HBCDD Serum LC-MS/MS (NR)	NA	Severe endometriosis [i]	NR (NR) NS[j]
den Hond et al. (2015)	NR Belgium	Cross-sectional NA	120 Adults (34.1, 30.0-–38.5) 100%	Total HBCDDs Serum GC-ECNI-MS (NR)	%> LOQ, cases 10 (11.8%), controls 5 (5.3%)	Subfertility	0.65 (0.14–2.92) 0.57[j]
Kim and Oh (2014)	NR South Korea	Cross-sectional NA	38 Infants (23–37) NR	α-HBCDD β-HBCDD γ-HBCDD Serum (Children) LC-MS/MS (0.036)	1.84 (< MDL–16.6); 0.46 (< MDL–1.3); 14.05 (< MDL–147.8)	Congenital Hypothyroidism (children)	NR (NR) NS
				β-HBCDD γ-HBCDD Maternal Serum LC-MS/MS (0.036)	0.46 (< MDL–1.88); 8.86 (< MDL–91.1)	Congenital Hypothyroidism (mothers)	NR (NR) NS
				α-HBCDD Maternal Serum LC-MS/MS (0.036)	2.57 (< MDL–13.8)	Congenital Hypothyroidism (mothers)	NR (NR) 0.004
Li et al. (2014)	2009 China	Cross-sectional NA	80 Adults (42, 30–50) 40%	α-HBCDD β-HBCDD γ-HBCDD ΣHBCDD Serum LC-MS (20, 20, 10)	5.9 (ND–2,702.5)	Thyroid hormones	NR (NR) NS[d]
Johnson et al. (2013)	2002–2003 USA	Cross-sectional NA	62 Adults (18–54) 100%	Total HBCDDs Dust GC-ECNI-MS	197 (IQR, 107–391)	Free androgen index	0.46 (NR) 0.004[d]
						Sex hormone binding globulin	–0.35 (NR) 0.03[d]
						Hormones[k]	NR (NR) NS[d]

Reference	Recruitment year Country	Study design Follow-up (years)	N Population group (age) (years) Gender (% males)	Compound Tissue analysed Method (LOD/LOQ)	Levels of Exposure (ng/g lipid)[a]	Endpoint health category	Effect estimate (95% CI), p-value
Kicinski et al. (2012)	2008–2011 Belgium	Cross-sectional NA	515 Adolescents (14.9 (0.7)) 53%	Total HBCDDs Serum GC-MS (30 ng/L)	< LOQ, (ND–234)	NES-CPT, Reaction Time[(n)]	−3.53 (−18.72–11.67) NS[(j)]
						NES-CPT, Errors of Omission[(n)]	27.80% (−17.5%–97.9%) NS[(l)]
						NES-CPT, Errors of Commission[(n)]	21.80% (−2.5% to 52.2%) NS[(l)]
						NES, System, Digit-Symbol test[(n)]	−0.44 (−6.59 to 5.72) NS[(j)]
						NES, Digit Span test[(n)]	0.13 (−0.22 to 0.49) NS[(j)]
Eggesbø et al. (2011)	2003 Norway	Cross-sectional NA	193 Neonates (3 days) 46%	Total HBCDDs Breast milk GC–ECNI-MS (NR)	0.54 (0.10–31)	TSH	0.00 (-0.02, 0.02) NS[(j)]
Weiss et al. (2006)	2000 Sweden	Cross-sectional NA	50 (25)[(m)] Adults (62, 52–81) 0%	ΣHBCDD α-HBCDD β-HBCDD γ-HBCDD Serum LC-MS/MS (0.12 pg/g; diastereomers 0.03, 0.06, 0.03)	0.46 (< 0.24–3.4)	Bone mineral density	NR (NR) NS
Stapleton et al. (2011)	2008–2010 USA	Cross-sectional NA	140 Adults (< 20: 23%) 0%	ΣHBCDD α-HBCDD β-HBCDD γ-HBCDD Serum LC-MS/MS (NR)	NA (no detected)	TSH, TT3, fT3, TT4, fT4	NA (NA) NA
Müller et al. (2016)	2012 Tanzania	Cross-sectional NA	150 Neonates (0) 0%	Total HBCDDs Breast milk GC-ECNI-MS (NR)	NA (1 sample above LOQ)	Birth weight Birth length	NA (NA) NA

BSID: Bayley Scales of Infant Development; CI: confidence interval. FU: follow-up; GC-ECNI-MS: Gas chromatography-electron capture negative ion-mass spectrometry; HBCDD: hexabromocyclododecane; IQR: interquartile range; LC-MS-MS: liquid chromatography-random mass spectrometry; LOD: limit of detection; LOQ: limit of quantification; MDI: mental development index; NA: not applicable; ND: not detected; NES-CPT: Neurobehavioral Evaluation System-Continuous Performance test; NS: not statistically significant.

(a): Median (range).
(b): Calculated as: Σ[food consumption (g/day) × food contamination (ng/g of food)]/bw (kg); food consumption over the previous year estimated through a validated 208-item semi-quantitative dietary questionnaire sent in 1993; food contamination data extracted from the 2nd Anses French Total Diet Study (TDS2) in 2011.
(c): HR for Quintile 2 vs. Quintile 1, Quintile 3 vs. Quintile 1, Quintile 4 vs. Quintile 1, Quintile 5 vs. Quintile 1, respectively.
(d): rho.
(e): Including motor performance (coordination, fine motor skills), cognition (intelligence, visual perception, visuomotor integration, inhibitory control, verbal memory and attention) and behaviour.
(f): Including verbal intelligence (WISC-III-NL); auditory-verbal memory (AVLT); selective visual attention ('Sky Search',TEA-Ch-NL); motor outcome (Movement-ABC).
(g): Including testosterone, sex hormone-binding globulin (SHBG); LH, FSH, estradiol (E2), free E2 (FE2) and inhibin B (InhB)], testes volume, penile length were also assessed.
(h): β-HBCDD and γ-HBCDD detection rates were lower than 75% and were excluded by the authors from the statistical analysis.
(i): Ovarian Endometrioma.
(j): OR or beta.
(k): Including follicle stimulating hormone (FSH), serum luteinising hormone (LH), estradiol, prolactin, free T4, total T3 and thyrotropin (TSH), inhibin B, testosterone; sex hormone binding globulin (SHBG), free androgen index (FAI), free unbound testosterone.
(l): % change.
(m): The PBDE/HBCDD extracts were later pooled (n = 25, 125 mL/pool) for stereoisomer determination.
(n): The Neurobehavioral Evaluation System (NES) is a computerised battery of tests developed to study neurobehavioural effects in humans. The Neurobehavioral Evaluation System-Continuous Performance Test (NES-CPT) evaluates sustained attention. The Neurobehavioral Evaluation System-Digit-Symbol Test evaluates a range of cognitive operations. The Neurobehavioral Evaluation System-Digit Span Test evaluates the working memory and the ability to hold and manipulate information. The Neurobehavioral Evaluation System-Finger Tapping Test evaluates the motor speed and coordination.

3.1.4. Mode of action

In the previous EFSA assessment (EFSA CONTAM Panel, 2011a), it was reported that HBCDDs increased liver weights and induced drug metabolism, mainly CYP2 and CYP3 and phase II conjugating enzymes, including thyroid hormone conjugating enzymes in the liver. The studies reviewed at the time indicated that HBCDD toxicity is mediated through activation of PXR and CAR-dependent pathways, with associated changes in gene expression of biotransformation enzymes with a LOEL of 3 and 30 mg/kg bw per day for female and male rats, respectively (Germer et al., 2006), and increased thyroid hormone glucuronidation ($BMDL_{10}$ 4.1 mg/kg bw per day[28]; van der Ven et al., 2006). These effects on gene expression and biotransformation in the liver were considered a feasible explanation for changes in thyroid and sex hormones, with downstream effects on neurodevelopment and reproduction (EFSA, 2011a). It was also indicated that '*neurodevelopmental effects might be associated with modulation of thyroid hormone homeostasis and the involved processes include direct interaction of HBCDDs with thyroid hormone receptors, and/or perturbations of thyroid hormone transport*.'

Since the previous EFSA Opinion further studies on the possible mode of action of HBCDDs have been published (see **Appendix E**) and the most relevant aspects for the risk assessment are described below.

3.1.4.1. Hepatotoxicity and metabolic effects

Farmahin et al. (2019) applied RNA sequencing to liver samples from male and female F344 rats exposed to HBCDDs (11% α-, 9% β- and 79% γ-HBCDD) at doses of 0, 250, 1,250 and 5,000 mg/kg diet (females: 0, 20, 102 and 430 mg/kg bw per day, males: 0, 19, 94 and 400 mg/kg bw per day) for 28 days. The expression of a total of 428 and 250 gene transcripts was altered by HBCDDs in males and females, respectively. The number of differentially expressed genes increased with HBCDD dose in both sexes. The three genes showing the largest change in expression were cytochrome P450 2B1 (*Cyp2b1*), nuclear receptor subfamily 1, group D, member 1 (*Nr1d1*) and metallothionein 1 (*Mt1*), all showing dose-dependent upregulation. Gene set enrichment analyses implicated PXR/CAR activation as the predominantly affected pathway, with genes associated with aryl hydrocarbon receptor (AHR) activation reaching significance in females, only, and no enrichment of PPARα, DNA damage or cytotoxicity gene categories. These data suggested that in the rat liver, HBCDDs activates the CAR and PXR, and the upregulation of *Mt1* indicates release of cytosolic free Zn^{2+}, which has also been observed in mouse brain and neural cells following HBCDDs exposure (Reffatto et al., 2018). The authors suggest that upregulation of *Mt1* is indicative of oxidative stress. This is a plausible interpretation because oxidative stress causes release of free Zn^{2+} through oxidation or nitrosylation of zinc-thiolate bonds, leading to a rise in free Zn^{2+}, activation of metal-responsive factor-1 (MTF1), which induces *Mt1* expression (Chung et al., 2006; Maret, 2011; Lee, 2018). PXR activation was specifically addressed in human HepG2 cells and rat H4IIE hepatoma cells, which were stably transfected with a reporter gene driven by PXR responsive elements (XREMs; Fery et al., 2010). HBCDDs did activate PXR in both human and rat cells, but only at a relatively high concentration of 15 μmol/L.

Benchmark Dose Modelling of RNA-sequencing data was carried out by Gannon et al. (2019b) using the rat liver data set by Farmahin et al. (2019, see above). The transcriptomic BMD (BMDt) modelling revealed a bimodal distribution of gene BMDs in both sexes with ranges of 66–104 mg/kg bw per day in males and 65–94 mg/kg bw per day in females. The most sensitive statistically significant pathway BMDt was LPS/IL-1 Mediated Inhibition of RXR Function, with BMDt (Benchmark Dose transcriptomics) of 66 and 71 mg/kg per day for males and females, respectively. To compare with apical effects in the same experiment, the BMD for increased liver weight in female rats was 99.5 mg/kg bw per day and the lowest BMD was 15.6 mg/kg bw per day for increased incidence of thyroid colloid depletion in males (Gannon et al., 2019b).

BMDt was also applied to transcriptomics responses in the liver were also investigated in male Sprague Dawley rats exposed to 0, 0.064, 0.64, 6.4, 64 and 640 mg/kg bw per day by gavage for five days (n = 6, age not reported; Shockley et al., 2020). There was as dose-dependent increase in gene expression with doses of 64 and 640 mg/kg bw per day resulting in differential expression (FDR < 0.05) of 23 and 247 genes, respectively. One differentially regulated transcript was found at each of the three lower doses. In this study, the BMDt was calculated as 38.11 mg/kg bw per day. Pathway analysis of regulated genes revealed enrichment of liver disease and metabolic pathways, including *Cyp2b6*, *Cyp3a5*, *Ces2c*, *Ugt2a1* and *Ugt2b17*, which all showed increased expression. There were no

changes in serum total T3 or T4, indicating that in this study an increased expression of biotransformation enzymes in the liver did not influence thyroid hormone levels.

Liver proteomics of HBCDDs exposure was studied in eu- and hypothyroid female Wistar rats, generated through a low iodine diet and sodium perchlorate supplementation, dosed HBCDDs at 0, 3 and 30 mg/kg bw per day HBCDDs for 7 days (Miller et al., 2016a). Internal concentrations of α- and β-HBCDD in adipose tissue were below the LOQ for the 3 mg/kg bw per day groups, whereas accumulation of γ-HBCDD (2–3 times) was noted in the highest dose groups. Liver weight was not affected by the 7-day exposure to HBCDDs, but the liver proteome profile was significantly altered with overall changes in abundance of 13 proteins in euthyroid females and 9 proteins in the hypothyroid animals. The proteins had mainly mitochondrial cytolocation and were involved in metabolic processes (gluconeogenesis/glycolysis, amino acid metabolism and lipid metabolism) and in oxidative stress responses in both eu- and hypothyroid rats. In contrast, eu- and hypothyroid Wistar rats, subjected to the same treatment regime, showed only very limited alterations of the liver proteome in males only, without any enriched functions or pathways (Miller et al., 2016b). Also, lower levels of γ-HBCDD accumulated in white adipose tissue of exposed male rats compared to females.

Dietary exposure to α-HBCDD for 28 days affected whole body lipid metabolism in juvenile female BALB/c mice, leading to changes in hepatic histology. The effects were aggravated in the presence of a diet rich in polyunsaturated fatty acids. Hepatic fatty acid profiling and gene expression analysis indicated that the dietary modulation of the hepatotoxic response to an α-HBCDD dose of 116 mg/kg bw per day was associated with differential effects on fatty acid β-oxidation (Bernhard et al., 2016). Oral exposure of male C57BL/6J mice once per week for 14 weeks (starting at 6 weeks of age) doses of HBCDDs of 0.35 and 0.7 mg/kg bw (equivalent to 0.05 and 0.1 mg/kg bw per day) in combination with a high-fat diet resulted in increased body and liver weight compared to controls on normal diet and to high-fat diet controls (Yanagisawa et al., 2014). These effects were not observed in animals exposed to HBCDDs in combination with a normal diet. At an HBCDD dose of 0.7 mg/kg bw per week in combination with high-fat diet, there was also an increase in liver triglyceride accumulation and steatosis along with increased expression of *Ppara* and *Pparg* as well as of the PPARγ target genes *Cd36*, *Fabp4* and *Fsp27*, indicating a plausible link to increased lipid droplet formation (Yanagisawa et al., 2014). An increase in *Pparg* mRNA expression was also observed in mice on a normal diet exposed to HBCDDs, but only the increase in *Cd36* was evident in animals fed high-fat diet without HBCDDs. These results suggest that HBCDDs increase lipid accumulation in tissues of mice fed a high-fat diet and that the effects are linked to increased expression and activity of PPARα and PPARγ. Xie et al. (2019) showed evidence suggesting that HBCDDs increase expression of *Pparg* and several of its transactivation target genes in epididymal white adipose tissue (eWAT) through reduced expression of *Wnt6*. WNT6 inhibits adipogenesis by suppression of *Pparg* expression via the canonical WNT/β-catenin signalling pathway (Cawthorn et al., 2012). This effect on adipogenic gene expression was also observed in male C57BL/6 mice (3–4 weeks old at the start of the experiment) exposed to HBCDDs at a weekly oral dose of 0.05 mg/kg bw (equivalent to 0.007 mg/kg bw per day) for 31 weeks with a normal chow diet and was associated with hypertrophy and weight of eWAT (Xie et al., 2019). Using mouse T3-L1 preadipocytes, it was further shown that HBCDDs reduced nuclearisation of β-catenin, providing mechanistic evidence for the putative link between reduced *Wnt6* and increased *Pparg* expression (Xie et al., 2019). The mouse PPARγ was not activated by HBCDDs in a reporter gene assay, suggesting that the PPARγ-mediated effect of HBCDDs is due to its stimulation of *Pparg* mRNA expression. Experiments on human preadipocytes from visceral adipose tissue (HPA-V) confirmed that HBCDDs increases lipid accumulation and expression of *LPL*, but an increased expression of *PPARG* mRNA could not be confirmed in human cells (Xie et al., 2019).

van den Dungen et al. (2017) found no lipid accumulation in HBCDD-exposed human preadipocytes (SGBS cells) and bone marrow-derived mesenchymal stem cells (MSC) induced to differentiate into adipocytes. However, the HBCDDs concentration required to induce lipid accumulation in the study by Xie et al. (2019) was higher (10 μmol/L) than the highest concentration used in this study (1 μmol/L) by van den Dungen et al. (2017). Osteoblastogenesis was induced in MSCs in the presence of 1 μmol/L HBCDDs (van den Dungen et al., 2017). WNT6 is a potent stimulator of MSC fate into osteoblasts (Cawthorn et al., 2012) and these results, therefore, seem inconsistent with those by Xie et al. (2019) showing reduced expression of *Wnt6 in vivo* and *in vitro* following HBCDD exposure.

A detailed study on HepG2 cells provided further insight into the mechanisms behind the effects of HBCDDs on lipid metabolism (Wang et al., 2016a), starting with NMR and following up with hypothesis driven experiments and analyses. HepG2 cells were exposed to high concentrations of a reagent-grade HBCDDs formula (purity: > 95%) at 50, 1,000 and 10,000 μg/L. Metabolomics analysis using NMR

revealed that HBCDDs exposure resulted in changes in amino acid metabolism, protein biosynthesis, fatty acid metabolism and phospholipid metabolism. β-oxidation of long chain fatty acids was suppressed, ATP production reduced, Na^+/K^+-ATPase inhibited and uptake of amino acids and glucose impaired. Reduced β-oxidation was accompanied by accumulation of free fatty acids and increased synthesis of phospholipids. The authors suggested that most effects may be a results of reduced ATP production leading to lowered of Na^+/K^+-ATPase and Ca^{2+}-ATPase activities, resulting in reduced glucose and amino acid uptake by cells (Wang et al., 2016a). In a related study, Wang et al. (2016b) treated female CD-1 mice by gavage for 28 days with 10 or 50 mg/kg bw per day of HBCDDs (95% purity; 10% α-, 10% β-, 80% γ-HBCDD) and used NMR and LC-MS/MS to study changes in the urinary metabolome. Effects were associated with TCA cycle, lipid metabolism, gut microbial metabolism and homoeostasis of amino acid. The TCA cycle was concluded to be particularly affected, supporting the results from studies in HepG2 cells indicating effects on energy metabolism (Wang et al., 2016a,b).

The cellular effects of HBCDDs were investigated on human hepatocyte cell line L02 (An et al., 2013). Cells were treated with a vast range of HBCDD concentrations (10^{-14}–10^{-4} mol/L) for 24, 48 or 72 h. Treatment with high concentrations (> 20 μmol/L) reduced the number of viable cells and increased apoptosis in a dose and time-dependent manner. On the contrary, lower concentrations of HBCDDs (10^{-13}–10^{-7} mol/L) for 72 h resulted in increased cell numbers and upregulated the proliferating cell nuclear antigen (PCNA) protein expression level. High concentrations of HBCDDs also caused significant decrease of mitochondrial membrane potential in L02 cells. ROS levels, as measured by the DCFH-DA probe, were also elevated in a concentration and time-dependent manner, with a significant increase observed after 96 h exposure to 10^{-13} mol/L of HBCDDs. Intracellular [Ca^{2+}] was statistically elevated already at 10 pM HBCDDs and increased concentration dependently up to the highest concentration of 60 μmol/L.

An increase in liver weight is a robust response observed in many *in vivo* studies with HBCDDs (EFSA CONTAM Panel, 2011a; Maranghi et al., 2013; Yanagisawa et al., 2014; Gannon et al., 2019a; Shockley et al., 2020). Zou et al. (2013) found that nanomolar concentrations of HBCDDs stimulated L02 hepatocyte proliferation in a DNA-dependent protein kinase, catalytic subunit (DNA-PKcs) dependent manner. This was associated with increased NRF2 protein expression and nuclear translocation of NRF2, leading to upregulation of its target gene *HO-1*. They further showed that activation of the NRF2-ARE pathway was dependent on the PI3K/AKT pathway in HBCDD exposed L02 cells.

In an investigation of the ability to induce an adaptive response to HBCDDs, L02 cells were pretreated for 48 h with nanomolar concentrations of HBCDDs followed by exposure for additional 48 h to high concentration of HBCDDs (50 μmol/L; An et al., 2016). Pretreatment with these low concentrations of HBCDDs protected against the subsequent high concentration of HBCDDs as shown by reduced loss of viable cells and ROS over-production. The compensatory response depended on the activation of the phosphatidylinositide 3-kinase/protein kinase B (PI3K/AKT) pathway, attenuated phosphorylation of adenosine monophosphate-activated kinase (AMPK signalling) and increased phosphorylation of p38 mitogen-activated protein kinases (p38 MAPK pathway) (An et al., 2016).

Cytotoxicity of HBCDDs in two different human hepatoma cell lines, L02 and HepG2, was found to be β-HBCDD > γ-HBCDD > α-HBCDD (Huang et al., 2016b). HepG2 cells exposed to 0, 10 and 100 nmol/L HBCDDs for 24 or 48 h without effects on viability (Zhong et al., 2015). The lower concentration stimulated migration and invasion of HepG2 cells, increased abundance of mammalian target of rapamycin (mTOR) and matrix metalloprotein 9 (MMP9) and suppressed E-cadherin (CDH1) expression. HBCDDs exposure further increased stimulatory phosphorylation of protein kinase B (pPKB/pAKT), extracellular signal-regulated kinase 32 (pERK) and p-p38 in a dose-dependent manner. Treatment of PI3K/AKT inhibitors LY294002 and MK-2206 effectively countered the increase of cell migration and invasion induced by HBCDDs. Together, these results indicate that the enhancement of cell migration and invasion was through activation of the PI3K/AKT/mTOR signalling pathway (Zhong et al., 2015). The same pathway also promoted autophagy in L02 cells exposed to 20 μmol/L HBCDDs (Jin et al., 2019).

Thus, studies published since the previous EFSA Opinion on HBCDDs (EFSA CONTAM Panel, 2011a) are generally consistent with effects on CAR and PXR in the liver of rodents. Several studies report high sensitivity of lipid metabolism and mitochondrial ATP production to HBCDD with downstream effects on Na^+/K^+-ATPase and Ca^{2+}-ATPase activities and a rise in intracellular [Ca^{2+}]. Effects on mitochondrial energy production are also consistent with increased ROS production and intracellular Zn^{2+} release, which has been reported in several studies. HBCDD increased adipogenesis in mice by suppressing WNT/β-catenin signalling, which resulted in increased expression of *Pparg* and its downstream adipogenic target genes. HBCDDs stimulated proliferation and migration of liver cell lines

at picomolar to nanomolar concentrations and there is evidence that these effects are related to activation of the PI3K/AKT/mTOR and oestrogen receptor (ER) signalling pathways. These effects might contribute along with activation of CAR, PXR and PPARs to the observed increase in liver weight in rodent studies with HBCDDs. A recent bioinformatics study used receptor-based pharmacophore models to identify effects of HBCDDs on hepatotoxicity and was able to predict effects on Wnt/ß-catenin, AMPK, PI3K/AKT and p38 MAPK pathways, among others, to generate oxidative stress, proliferation and inflammation, supporting some of the empirically derived based of evidence for hepatotoxicity of HBCDDs (Dai et al., 2020).

3.1.4.2. Endocrine related effects

Parallel transcriptomics profiling of mouse brains and two neuronal cell lines (N2A and NSC-19) exposed to HBCDDs (see **Section 3.1.4.2**) strongly implicated effects on the hypothalamus–hypophysis–gonadal axis because the overall most significant predictors of upstream regulators of the observed gene expression profiles were gonadotropin release hormone, 17β-oestradiol, dihydrotestosterone and prolactin (Reffatto et al., 2018). The fact that these effects were observed not only *in vivo* but also in two different neuronal cell lines is suggesting direct effects on sex steroid target tissue. HBCDDs have showed both anti-androgenic and anti-oestrogenic activity in CALUX® sex steroid receptor reporter gene assays and MCF-7 cell proliferation assays (Hamers et al., 2006; Dorosh et al., 2010; Krivoshiev et al., 2016).

The potential mode of action of reproductive toxicity of HBCDDs (95% pure) was tested *in vitro* in Leydig cells of peripubertal (51 days old Wistar) rats during 6 h exposure. Cells were exposed to 0, 1, 2, 5 and 10 μmol/L HBCDDs in the absence or presence of hCG (human chorionic gonadotropin) (0.125, 0.25, 1 and 10 ng/mL). Treatments in the presence of 20 μmol/L cholesterol or 20 μmol/L 22 (R)-hydroxycholesterol were performed to localise the step in the steroidogenic process that is affected by HBCDDs. Opposite effects of HBCDDs on steroidogenesis were observed in presence or absence of hCG, in absence of hCG, a concentration dependent increase in androgen and progesterone levels was observed in culture medium. However, in presence of hCG (1–10 ng/mL), HBCDDs had no effect and in presence of low hCG (0.125–0.25 ng/mL), HBCDDs significantly decreased androgen production in a concentration-dependent manner. Inhibition of progesterone was also observed in HBCDD treated cells in presence of hCG. HBCDDs inhibited human chorionic gonadotropin- and forskolin-supported cAMP accumulation and steroidogenesis. HBCDDs inhibited also basal cAMP production in presence or absence of hCG, but elevated basal steroidogenesis. Expression of several cAMP-dependent genes, including steroidogenic acute regulatory protein, cholesterol side chain cleavage enzyme and 3-hydroxysteroid dehydrogenase was also suppressed by HBCDDs, but this was not accompanied by a decrease in steroidogenic acute regulatory protein expression. HBCDDs caused significant decrease in mitochondrial membrane potential in untreated and human chorionic gonadotropin-treated cells. The authors concluded that HBCDDs effect on steroidogenesis in Leydig cells reflect changes in mitochondrial membrane potential-dependent ATP production, which secondarily reduced cAMP production and basal and cAMP-regulated cholesterol transport. This in turn facilitates basal but inhibits cAMP-dependent steroidogenesis (Fa et al., 2013, 2015). Further experiments showed that HBCDD exposure of Leydig cells caused inhibition in the expression of genes for steroidogenic enzymes, luteinising hormone receptor, regulatory and transport proteins and a decrease in abundance of StAR (Fa et al., 2015). Enzymatic assays indicated loss of activities of (CYP11A1) and 17β-hydroxysteroid dehydrogenase (HSD17β), indicating diminished capacity to synthesise progesterone and testosterone, respectively. The authors concluded based on these data that conversion of 22-OH cholesterol to pregnenolone and of progesterone to testosterone may be the sites of HBCDD action on steroidogenesis in Leydig cells (Fa et al., 2015).

Exposure of human MCF-1 breast cancer-derived cells to HBCDDs at concentrations above 10 μmol/L increased proliferation in an oestrogen-dependent manner (Dorosh et al., 2010). Oestrogenic effects of HBCDDs were further assessed by measuring expression of the oestrogen-regulated trefoil factor 1 (*TFF1*) gene with effects starting at 200 nM. Both the cell proliferation and gene expression responses were blocked by the anti-oestrogen, ICI 182,780, suggestive of an oestrogen receptor mediated response.

Changes in thyroid hormone homeostasis have been noted in numerous studies of animals orally exposed to HBCDDs (see **Section 3.1.2.2**). Miller et al. (2016a) studied the mechanism of HBCDD-induced thyroid and liver (see above) toxicity. Eu- and hypothyroid female Wistar rats were fed 0, 3 and 30 mg/kg bw per day HBCDDs for 7 days. Hypothyroidism was successfully achieved with an increase in plasma concentration of TSH, a decrease in plasma total T3 and free T3. Exposure over

7 days to HBCDDs did not affect plasma thyroid hormones, TSH, leptin or corticosterone concentrations. However, the increase in TSH concentration was positively correlated with the internal γ-HBCDD concentration in eu-thyroid rats, but not in hypothyroid rats. In male rats, subjected to the same experimental paradigm, corticosterone concentrations increased in unexposed hypothyroid animals and tended to increase further with HBCDD exposure, however, HBCDD exposure had no effect on serum leptin concentrations (Miller et al., 2016a).

Thyroid hormones are important for brain development and evidence from two related *in vitro* studies indicate that HBCDDs might be a direct interruptor of these processes locally in the rat cerebellum. A very low HBCDDs concentration of 0.1 nM suppressed thyroid hormone-induced dendritic branching of Purkinje cells in primary cerebellar cultures (Ibhazehiebo et al., 2011a). Thyroid hormone-stimulated neurite extension of granule cell aggregate cultures was also attenuated by 0.1 nM HBCDDs (Ibhazehiebo et al., 2011b). Brain-derived neurotrophic factor (BDNF) is expressed in response to thyroid hormone receptor activation and is stimulates granule cell migration and neurite extension. Addition of BDNF to the culture medium completely rescued HBCDDs inhibition of neurite extension indicating that the effect might be caused by HBCDDs blocking thyroid hormone-stimulated increase in BDNF (Ibhazehiebo et al., 2011b). Using a thyroid hormone reporter gene assay it was found that the same concentration of HBCDDs (0.1 nM) that had effects on dendritic branching and neurite extension reduced thyroid hormone receptor-mediated transcription (Ibhazehiebo et al., 2011a). These studies indicate that HBCDDs may block thyroid hormone mediated transcription and associated developmental processes in the brain.

Extrapolation of effects on thyroid hormone homeostasis observed in rodents to humans is complicated by differences in levels and binding capacity of transporting proteins and feedback regulation of thyroid hormone homeostasis. In humans and animals, thyroid hormones circulating in the blood are degraded by stepwise deiodination, sulfation or glucuronidation. It was shown that sulfation of T3 and T4 in rats and mice is less than in humans and that glucuronidation predominates in rats (Bartsch et al., 2018). So, even, if rodents are more sensitive than humans to perturbations of thyroid hormone homeostasis, rodent data on the effects of HBCDDs on thyroid hormone levels or signalling might be of relevance for human health risk assessment. Moreover, the human fetus and neonate has a shorter T4 half-life than adults which may make them more vulnerable to a lowering of serum T4 levels (Li et al., 2019a). Therefore, it is difficult to dismiss a relevance for humans of the thyroid hormones insufficiency observed in the rat as a result of induction of hepatic metabolism.

Effects on insulin and glucose homeostasis have also been observed at low doses of HBCDD exposure. Male C57BL/6J mice were dosed with HBCDDs by gavage for 14 weeks (starting at 6 weeks of age) at doses of 0.35 and 0.7 mg/kg bw per week (equivalent to 0.05 and 0.1 mg/kg bw per day) in combination with either a normal or high-fat diet (Yanagisawa et al., 2014). The high-fat diet by itself did not increase random blood glucose but did raise random blood insulin levels. Mice exposed to HBCDDs at a dose of 0.7 mg/kg bw per week in combination with the high-fat diet had lower insulin sensitivity and higher random blood glucose levels than the mice on the high-fat diet alone, suggesting an effect of HBCDDs. There were however no clear HBCDD-dependent effects in oral glucose tolerance or insulin sensitivity tests. Mice on the high-fat diet that were exposed to HBCDDs at dose of 0.7 mg/kg bw per week also showed reduced expression of *Glut4* in epididymal adipose tissue (Yanagisawa et al., 2014).

Thus, HBCDDs may interfere with synthesis of sex steroids hormones both by interfering with cAMP-dependent cholesterol uptake and by causing dysregulation of enzymes involved in sex steroid metabolism. However, there are also evidence that HBCDDs alter the response of tissues to sex steroids. Cell culture experiments indicate that whilst HBCDD exposure does not remarkably change thyroid hormone levels, thyroid hormone mediated gene expression and downstream functions in neurons are exceptionally sensitive to HBCDDs and that this might contribute to the observed effects of HBCDDs on neural function in animal studies. Relatively low doses of HBCDDs cause glucose dyshomeostasis, with increases in random blood insulin and glucose, and these effects are exacerbated in combination with a high-fat diet.

3.1.4.3. Oxidative stress

Expression of four DNA repair genes was investigated after exposure of human breast cells HBL-100 to HBCDDs at 5, 10 or 50 mg/L (7.8, 16 and 78 μM) (Li et al., 2017b). The expression of *ATM*, a key regulator of the DNA repair response, was dose-dependently induced by HBCDD. DNA breaks levels correlated positively with ROS and *ATM*, but a negative correlation was present with *OGG1* and *MTH1*.

Excessive production of ROS has been reported in a variety of studies on HBCDD toxicology. The mechanism(s) involved has/have not been demonstrated but are plausibly related to impairment of

mitochondrial energy production as discussed in previous sections. Oxidative stress from HBCDD exposure has also been suggested through toxicological network analysis based on the predicted HBCDD targets HSP90AA1, AR, RORA and MET (Dai et al., 2020).

3.1.4.4. Neurotoxicity

In its previous Opinion on HBCDDs, the CONTAM Panel concluded that effects of HBCDDs on behaviour may have been caused by changes in thyroid hormone levels, brought about by an increased glucuronidation of thyroid hormone in the liver (EFSA CONTAM Panel, 2011a). An increased T4 UGT activity in the liver was observed in rats with a $BMDL_{10}$ of 4.1 mg/kg bw per day[28] (corresponding to a LOAEL of 100 mg/kg bw per day as identified by the CONTAM Panel using ANOVA HSD post hoc test) and this was associated with an increased expression of PXR and CAR-dependent biotransformation enzymes (Germer et al., 2006; van der Ven et al., 2006). However, effects on thyroid hormone concentrations and the thyroid gland have only been observed at doses $\geq$ 20 mg/kg bw per day, which is at least an order of magnitude higher than those reported to have effect on neurodevelopment (**Tables 9** and **10**). It is therefore unlikely that neurodevelopmental deficiencies were secondary to changes in thyroid hormone metabolism and levels.

HBCDDs can pass the blood brain barrier, accumulate in the brain (Covaci et al., 2006; Rasinger et al., 2014) and cause neurotoxicity in juveniles at relatively low doses (Eriksson et al., 2006; Lilienthal et al., 2009; Zhang et al., 2017a) (see **Section 3.1.2**; EFSA CONTAM Panel, 2011a). Rodents exposed to HBCDDs during early development showed dose-dependent changes in spontaneous behaviour (hypoactivity followed by hyperactivity in mice, Eriksson et al., 2006), impaired hippocampus-based spatial memory and learning (Eriksson et al., 2006; Zhang et al., 2017a). Effects on dopamine-dependent behaviour and auditory evoked potentials were observed in rats gestationally exposed to HBCDDs (Lilienthal et al., 2009).

Genskow et al. (2015) and Pham-Lake et al. (2017) investigated effects on dopaminergic neurons in the striatum and hippocampus, respectively, of adult male C57BL/6J mice given HBCDDs at 25 mg/kg bw per day by gavage for 30 days. In both brain areas they found reduced expression of dopamine transporters *Dat* and *Wmat2*, which are involved in clearing the synapse from dopamine and reincorporating into presynaptic vesicles. HBCDDs were found to inhibit dopamine uptake with a half maximal inhibitory concentration (IC50) of 4 μM (Mariussen and Fonnum, 2003). In the hippocampus, there was in addition reduced expression of proteins involved in synthesis (tyrosine hydroxylase) and degradation (catechol-O-methyltransferase and monoamine oxidase-B) of dopamine (Pham-Lake et al., 2017). These studies are supportive of effects of HBCDDs on presynaptic dopaminergic neurons.

Dietary gestational exposure of Sprague-Dawley rats to HBCDDs resulted in an increased number of mature neurons and apoptotic bodies in the hilus region of the hippocampus in pups (Saegusa et al., 2012). Doses were not reported, but these effects were observed in pups of dams given HBCDDs at 10,000 mg/kg feed and were absent in the two lower HBCDD dietary concentrations of 100 and 1,000 mg/kg feed. Experiments of neuronal cell lines (N2A, NSC19, SH-SY5Y) have confirmed that exposure to HBCDDs causes cell death by apoptosis at about 1 μM concentration (Reffatto et al., 2018; Shi et al., 2020). Szabo et al. (2017) adopted the experimental paradigm of Eriksson et al. (2006), giving a single oral dose on PND10 of 3, 10 or 30 mg/kg bw of either α-HBCDD or β-HBCDD, or 30 mg/kg bw of an HBCDD mixture. Metabolomic analysis of the serum collected four days after exposure showed dose-dependent reduction in glutamate, which was statistically significant at 10 mg/kg bw.

Rasinger et al. (2014) exposed juvenile Balb/c mice via the diet to HBCDDs at 199 mg/kg bw for 28 days, resulting in an HBCDD accumulation in the brain of 4.7 μg/g dw, and reported transcriptomic and proteomic profiles signifying neurological disease with specific effects on Ca^{2+} and Zn^{2+} signalling processes. These effects persisted at a lower dose of 49.5 μg/kg bw per day from the same experiment, which were published in a separate report (Rasinger et al., 2018). Similarly, a study carried out in parallel in brain of female BALB/c mice exposed to 199 mg/kg bw per day of HBCDDs via the diet for 28 days and *in vitro* for 24 h at 1 and 2 μM in two mouse cell lines (N2A and NSC-19) of neuronal origin implicated Ca^{2+} and Zn^{2+} dyshomeostasis in glutamatergic neurons as prominent upstream regulators of transcriptomics profiles both *in vivo* and *in vitro* (Reffatto et al., 2018). Follow-up experiments in primary glutamatergic hippocampal neurons showed that HBCDD exposure (1 and 2 μM) inhibited glutamate-dependent as well as Zn^{2+}-evoked intracellular Ca^{2+} release (Reffatto et al., 2018). HBCDDs also triggered intracellular Zn^{2+} release, an effect which was partially caused by oxidative stress. Oxidative stress responses were also substantiated by transcriptomics and proteomics profiles (Rasinger et al., 2018; Reffatto et al., 2018). Both pre- and post-synaptic events are dependent on Ca^{2+} signalling in glutamatergic neurons and many glutamatergic neurons, particularly

in the hippocampus, release Zn^{2+} along with glutamate to modulate responses by N-methyl-d-aspartate (NMDA) receptors (Sensi et al., 2011) and AMPA receptors (Kalappa et al., 2015). The NMDA receptor has a GluN2A subunit which has a high affinity Zn^{2+}-binding site that mediates allosteric inhibition of post-synaptic Ca^{2+} currents (Sensi et al., 2011). Additionally, in hippocampal neurons, Zn^{2+} can evoke post-synaptic currents via activation of GPR39, which is a metabotropic zinc receptor that stimulates Ca^{2+} release in cells (Besser et al., 2009).

Intracellular release of Ca^{2+} following exposure to HBCDDs has been evidenced in other cell types. Exposure of PC12 cells to 2 μM HBCDDs inhibited depolarisation-evoked intracellular [Ca^{2+}] transients and associated catecholamine release (Dingemans et al., 2009). Likewise, rat H9C2 cardiomyocyte cells treated with 2–200 nM HBCDDs showed reduced the cytosolic free Ca^{2+} concentrations and increased Ca^{2+} accumulation in the sarcoplasmic reticulum in H9C2 rat cardiomyocytes (Wu et al., 2016b). HBCDDs were also reported to inhibit the sarcoplasmic-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) in neuroblastoma SH-SY5Y cells by preventing its ATP binding with an IC50 of 2.7 μM (Al-Mousa and Michelangeli, 2014). The relative cytotoxicity of HBCDD diastereoisomers in SH-SY5Y cells was ß- > γ- > α-HBCDD and this order was mirrored in the potency of the respective diastereoisomer in generating ROS (Shi et al., 2019).

Ibhazehiebo et al. (2011a,b) found that an exceptionally low HBCDD concentration of 0.1 nM was sufficient to suppress thyroid hormone-induced dendrite branching of Purkinje cells and extension of granule cell aggregate in primary cerebellar cultures from rat neonates. As these are endocrine-related effects, they will be discussed further below in **Section 3.1.4.3**. Possible thyroid hormone related effects on the nervous system were also indicated in a study in male rats maternally exposed from GD10 to PND20 via dams given HBCDDs at 100, 1,000 or 10,000 mg/kg in the diet (corresponding to approximately 12, 120 and 1,200 mg/kg bw per day; Fujimoto et al., 2013). Pups sampled at PND20 showed an increased abundance of ret proto-oncogene and vimentin positive cells in the white matter of the cingulum, suggesting an increased number of immature oligodendrocytes. This could potentially be interpreted as an effect of low thyroid hormone as developmental hypothyroidism has been reported to produce a similar effect (Fujimoto et al., 2012). No remarkable effects on thyroid hormone levels were observed in pups following maternal HBCDD exposure, which might mean that the effect is brought about by a reduced responsiveness of the thyroid hormone receptor (Fujimoto et al., 2013) as observed in cell culture (Ibhazehiebo et al., 2011a,b).

Thus, mechanistic studies on the effects of HBCDDs on the nervous system support specific effects on dopaminergic and glutamatergic neurons. This appears to include inhibition of dopamine and glutamate reuptake following synaptic release, and deregulation of Ca^{2+} and Zn^{2+} which are both integral to the function of glutamate transmission. There is also *in vitro* evidence suggesting that thyroid hormone mediated developmental processes in the brain could be affected by HBCDDs through direct effects on developing neurons on their ability to respond to thyroid hormone.

3.1.5. Consideration of critical effects and dose–response analysis for the human risk assessment

3.1.5.1. Consideration of critical effects

In its previous Opinion on HBCDDs, the CONTAM Panel noted that most of the toxicological studies were performed with HBCDD preparations for which the purity and stereoisomer composition was not always indicated. Therefore, a risk assessment of individual stereoisomers was not possible (EFSA CONTAM Panel, 2011a). The CONTAM Panel concluded that the main targets for toxicity were the liver, thyroid hormone homeostasis and the reproductive, nervous and immune systems. The most sensitive endpoints for HBCDDs were considered to be an increased thyroid weight in female rats from a repeated dose 28-day study with a $BMDL_{10}$ of 1.6 mg/kg bw per day as calculated by the authors (van der Ven et al., 2006), and behavioural effects in male mice from a study with a single HBCDD administration at PND10 (Eriksson et al., 2006) with a $BMDL_{10}$ of 0.93 mg/kg bw calculated by the CONTAM Panel at that time. The effect on the thyroid was concluded to originate from increased T4 glucuronidation in the liver, leading to a decrease in blood T4 concentration (van der Ven et al., 2006). Changes in thyroid hormone homeostasis were considered to be associated with the neurodevelopmental deficiencies caused by HBCDD exposure (EFSA CONTAM Panel, 2011a). In its 2011 Opinion, the Panel expressed concerns about the reliability of the $BMDL_{10}$ of 1.6 mg/kg bw per day, as the dose-response was not clear (at doses up to 30 mg/kg bw, except at the dose 1 mg/kg bw the increase was less than 6%), and decided to use the $BMDL_{10}$ of 0.93 mg/kg bw for behavioural

effects in male mice (Eriksson et al., 2006) as the Reference Point for the hazard characterisation (see **Section 1.3.5** on previous risk assessments).

The new studies published since then have still in most cases been conducted with HBCDDs with no information on the stereoisomer composition specified. One study was performed with an HBCDD mixture enriched with α-HBCDD (81%) (Gannon et al., 2019a), and two studies were performed with α-HBCDD alone (Maurice et al., 2015; Bernhard et al., 2016). These studies confirmed that in 28-day studies HBCDD exposure primarily resulted in liver and endocrine related effects.

In the liver, increased weight and hepatocellular hypertrophy were observed at doses ≥ 20 mg/kg bw per day in 28-day studies in rats and mice (Maranghi et al., 2013; Rasinger et al., 2014, 2018; Bernhard et al., 2016; Gannon et al., 2019a). These effects could be due to increased adipogenesis due to induced PPARgamma expression (see **Section 3.1.4.1**). Liver lesions, such as increased vacuolation in hepatocytes, increased pyknotic nuclei, lymphocytic infiltration and hyperaemic vessels, were seen at doses 49.5 μg/kg bw per day and 199 mg/kg bw per day in a 28-day mice study (Rasinger et al., 2018). However, these liver lesions were not considered further because there was no difference in the incidence of the lesions between these doses varying by three orders of magnitude.

Changes in the thyroid observed in the new studies were increased weight, follicular hypertrophy, hyperplasia and/or colloid depletion. In contrast to the studies available at the time of the previous CONTAM Opinion on HBCDDs (EFSA CONTAM Panel, 2011a), newer studies reported effects on the thyroid only at doses ≥ 20 mg/kg bw per day. Since changes in thyroid hormone levels have also only been observed at doses ≥ 20 mg/kg bw per day, changes in the thyroid gland and circulating thyroid hormone concentrations were not considered for the establishment of a Reference Point.

Changes in testosterone (at a dose of 199 mg/kg bw per day) and oestradiol (not dose-related) concentrations in mice and reduction in the number of growing ovarian follicles in rats (at doses ≥ 20 mg/kg bw per day) were also observed.

Two studies in male C57BL/6 mice exposed to HBCDDs by oral gavage once per week provided evidence of effects on lipid and sugar metabolism at low doses (Yanagisawa et al., 2014; Xie et al., 2019). Yanagisawa et al. (2014) found dose-dependent increases in body and liver weights at HBCDD doses of 0.035 and 0.70 mg/kg bw per week (5 and 100 μg/kg bw per day) when given in combination with a high-fat diet. There were also increases in blood glucose and insulin levels and microvesicular steatosis and macrophage accumulation in adipose tissue in the high-dose group, only. However, as these effects were only observed in combination with a high-fat diet, they were not considered relevant for establishment of a Reference Point. Effects on lipid metabolism were also found in a study exposing the same mouse strain to a weekly HBCDDs dose of 0.050 mg/kg bw, corresponding to 7.1 μg/kg bw per day (Xie et al., 2019). Increased epididymal white adipose tissue weight and hypertrophy was observed and mechanistic evidence suggested that the adipogenic effects in mice was due to increased expression of *Pparg* mRNA. However, whilst HBCDD-induced lipid accumulation in human preadipocytes, the adipogenic effect appeared less potent than that in mice and did not involve stimulation of *PPARG*-mRNA expression (Xie et al., 2019). The CONTAM Panel noted that both studies (Yanagisawa et al., 2014; Xie et al., 2019) administered only one dose level, that the relevance to human health of the effects reported by Xie et al. (2019) is unclear, and that for Yanagisawa et al. (2014), the effects were observed in combination with a high-fat diet. Because of these considerations, these studies were not considered further for the derivation of a Reference Point.

No new developmental and reproductive study has been identified since the previous EFSA assessment. In the previous assessment (EFSA, 2011a), several reproduction toxicity studies in rats reported reduced fertility index (at 115 mg/kg bw per day), reduction of the number of ovarian primordial follicles (138 mg/kg bw per day), decrease in testes weight ($BMDL_5$ of 11.5 mg/kg bw per day[28]), increased anogenital distance in male pups ($BMDL_{10}$ PND4: 95.6 mg/kg bw per day[28]), and delayed vaginal opening ($BMDL_{10}$: 82.2 mg/kg bw per day[28]) (Ema et al., 2008; van der Ven et al., 2009). No fetotoxicity, embryotoxicity or teratogenic effects were reported in developmental toxicity studies in rats (up to dietary doses of 750 mg/kg bw per day from GD0–20 or up to 1,000 mg/kg bw per day by gavage from GD6–9) (Murai et al., 1985; Stump, 1999; EFSA CONTAM Panel, 2011a). Increased pup mortality during lactation was noted in the F2 generation at 1,008 mg/kg bw per day in males and at 1,363 mg/kg bw per day in females) (Ema et al., 2008).

Regarding the immune system, the rodent studies published since the previous EFSA assessment confirm the effects, such as decreased weight and increased lesions in the thymus, decreased splenocytes proliferation and increased spleen lesions, decreased T-cell and increased B-cell

populations, increased NK-cells, decreased serum IgA or IgG immunoglobulin (see **Section 3.1.2.4**). These effects were observed at doses around 20 mg/kg bw per day in F344 rats.

In the previous EFSA assessment (EFSA CONTAM Panel, 2011a), several studies in rodents were identified demonstrating that exposure to HBCDDs induced neurodevelopmental effects on behaviour after single (Eriksson et al., 2006) or repeated administration (Ema et al., 2008; Lilienthal et al., 2009; Saegusa et al., 2009). The study by Eriksson et al. (2006), in which HBCDDs (3% α-, 8% β- and 89% γ-HBCDD) were administered as a single dose by gavage on PND10 at 0.9 or 13.5 mg/kg bw, provided the lowest doses leading to neurobehavioural effects. PND10 was selected by Eriksson et al. (2006) as the optimal day for administering the HBCDDs because it represents a critical period in the development of the rodent brain, coinciding with a period of rapid brain growth spanning the first 3–4 weeks of postnatal life and reaching its peak at around day 10 (Gaitonde and Richter, 1966; Davison and Dobbing, 1968; Eriksson et al., 1992, 2000; Ahlbom et al., 1995; Eriksson and Fredriksson, 1998). They stated that in humans this period begins in the third trimester of pregnancy and continues throughout the first 2 years of life. A dose-dependent decrease in spontaneous behaviour (horizontal locomotion, rearing and total activity) was noted (LOAEL of 0.9 mg/kg bw). Impaired spatial learning and memory were observed in mice exposed to 13.5 mg/kg bw HBCDDs (NOAEL of 0.9 mg/kg bw). In its 2011 Opinion, the CONTAM Panel had noted limitations and concerns regarding the single administration protocol and that the litter effect was not taken into account properly, and recommended that the results required independent verification (EFSA CONTAM Panel, 2011a). However, the CONTAM Panel also noted at that time that PND10 covered a relevant neurodevelopmental period in experimental animals, and that it provided the lowest dose leading to neurobehavioural effects. Thus, in its previous assessment the CONTAM Panel decided to perform dose–response modelling on the results obtained in this study, and used the lowest and most reliable BMDL (for horizontal locomotion) as the Reference Point for the hazard characterisation of HBCDDs (EFSA CONTAM Panel, 2011a).

Of the new studies published since the previous assessment on effects on the nervous system, most did not investigate neurobehavioural effects but provided cellular and molecular support for effects on glutamatergic and dopaminergic neurons in the hippocampus and striatum (Saegusa et al., 2012; Rasinger et al., 2014, 2018; Genskow et al., 2015; Pham-Lake et al., 2017; Reffatto et al., 2018). Only one study provided information on α-HBCDD (Maurice et al., 2015), showing significant effects at a very low dose of 22 ng/kg bw per day, but not at the higher dose of 66 ng/kg bw per day for motor activity and anxiety in offspring of female rats dosed by gavage from GD0 to PND21. Miller-Rhodes et al. (2014) administered HBCDDs to pregnant rats and found significant changes at all doses but no clear dose–response relationship in multiple neurobehavioural tests performed on the offspring at ages up to 21 months. The study of Zhang et al. (2017a) investigated the effects of HBCDDs (0.3, 3 and 30 mg/kg bw per day) on spatial learning and memory after exposure of rats from PND10 to PND70. Effects were observed at all doses. This study is supportive of the Eriksson et al. (2006) study in that HBCDDs induced neurobehavioural effects, but it was not considered further for the derivation of a Reference Point due to limitations in the study.

The current assessment highlighted additional limitations in the study of Eriksson et al. (2006), including possible alternative explanations/drivers for the observed changes in spontaneous behaviour that were not assessed, and that the mice were only tested when adults so key adaptations across adolescence, for example, could have altered their behaviour as adults. However, the CONTAM Panel concluded that these results could not be discounted, and the LOAEL was determined to be 0.9 mg/kg bw, based on changes in spontaneous behaviour (horizontal locomotion, rearing and total activity). Due to limitations in the assessment of spatial learning and memory, this endpoint is not considered suitable for the establishment of a Reference Point in the current assessment.

The new *in vitro* genotoxicity studies available do not change the previous conclusion that HBCDDs are not genotoxic *in vitro* or *in vivo*. The slight induction of DNA strand breaks observed in some *in vitro* tests is most likely due to oxidative stress.

In the previous Opinion, it was stated that '*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*'. No new carcinogenicity studies have been identified since the previous Opinion. Based on the available information to the CONTAM Panel, i.e. the lack of carcinogenicity in the mouse study, the lack of direct genotoxicity and the information available on mode of action, there is no indication that HBCDDs are carcinogenic.

Concerning effects in humans, since the previous EFSA assessment fifteen new epidemiological publications were identified assessing the association between exposure to HBCDDs and several human health endpoints. Neurodevelopment and thyroid dysfunction were assessed in children, while in adults, subfertility, type 2 diabetes, severe endometriosis and ovarian endometrioma and breast cancer metastasis were assessed. In the single available prospective study using measures of internal exposure, statistically significant – yet opposite-direction – results were reported for endpoints related to neurodevelopment (coordination, and WISC-III total and verbal intelligence). No concordant findings were identified in the single available cross-sectional study on the same research question (Kicinski et al., 2012). In another large longitudinal study in a European population assessing the association between HBCDDs and type 2 diabetes, statistically significant results were reported but there was no assessment of internal exposure (Ongono et al., 2019). Among the available cross-sectional studies, statistical significance was recorded for the associations between lower α-HBCDD concentrations and the risk of having a child born with congenital hypothyroidism (Kim and Oh, 2014), and between HBCDD levels and free androgen index and sex hormone binding globulin (Johnson et al., 2013). Although the number of epidemiological studies has grown in the last years, limitations related to exposure assessment, study design, sample size, effects direction and lack of validity hinder the possibility to consider any of the assessed endpoints as the basis for the risk assessment.

Neurodevelopmental effects on behaviour are supported by mechanistic studies with HBCDDs, indicating specific effects on dopaminergic and glutamatergic neurons, which could be the mode of action of the neurobehavioural effects. These include inhibition of dopamine and glutamate reuptake following synaptic release, and deregulation of Ca^{2+} and Zn^{2+} which are both integral to the function of glutamate transmission. There is also *in vitro* evidence suggesting that thyroid hormone mediated developmental processes in the brain could be affected by HBCDDs through direct effects on developing neurons in their response to thyroid hormones (see **Section 3.1.4.2**).

Overall, the CONTAM Panel concluded that the neurodevelopmental effects on behaviour still can be considered the critical effect for the risk characterisation.

3.1.5.2. Dose–response analysis

Since the evidence from the available human data was not sufficient to base the risk assessment on, the CONTAM Panel considered the data from studies on experimental animals to identify Reference Points for the human hazard characterisation.

The Panel performed BMD modelling according to the EFSA Guidance on the use of the BMD approach in risk assessment (EFSA Scientific Committee, 2017) for the data on horizontal locomotion, rearing and total activity in mice from Eriksson et al. (2006). Since this study was performed with a mixture of HBCDD stereoisomers, a dose–response analysis for individual stereoisomers was not possible. The results of the modelling are summarised in **Table 12** and details of the BMD analysis are reported in **Annex C**.

Table 12: Benchmark dose (BMD) modelling for the effects of HBCDDs in Eriksson et al. (2006) with a benchmark dose response (BMR) of 10%

Critical effect	Model	$BMDL_{10}$	$BMDU_{10}$	Reference
		(mg/kg bw per day)		
Horizontal locomotion	Model averaging	0.08	2.09	Eriksson et al. (2006)
	Lowest	0.05	2.15	
Rearing	Model averaging	0.09	0.76	
	Lowest	0.06	0.75	
Total activity	Model averaging	0.58	8.41	
	Lowest	0.51	12.7	

Note: Detailed results of the BMD modelling can be found in **Annex C**.

In its previous Opinion on HBCDDs (EFSA CONTAM Panel, 2011a), the CONTAM Panel performed dose–response analysis and the calculation of the BMD and the BMDL of the Eriksson et al. (2006) study were based on the previous EFSA guidance on the use of the BMD approach in risk assessment (EFSA, 2009). Re-analysis of the Eriksson et al. (2006) data with the new EFSA guidance for BMD modelling (EFSA Scientific Committee, 2017), led to wider intervals around the BMD. This is mainly due to differences in methods recommended by the two guidance documents. In the current guidance, for

continuous data only four models are used, and only two models per nested family (Hill and exponential). Moreover, the new BMD guidance (EFSA Scientific Committee, 2017) does not recommend constraining the steepness/shape parameter in the models. Therefore, if the shape of the dose–response curve is not sufficiently constrained by the data itself in the region of the BMR (e.g. due to the low number of dose groups, and/or the dose spacing, and/or limited sample size) a large BMD confidence interval can result as a consequence. The CONTAM Panel noted that the BMDLs for horizontal locomotion and rearing are far below the lowest dose administered. Overall, the Panel decided to identify the Reference Point based on the NOAEL/LOAEL approach instead of BMD modelling. From the Eriksson et al. (2006) study, a LOAEL of 0.9 mg/kg bw was identified for spontaneous behaviour.

Daily exposures to HBCDDs result in increasing levels in the body. For this reason, the accumulated concentrations in the body or body burden, rather than the daily exposure, should be considered as the proper starting point for the risk assessment. In mice (Eriksson et al., 2006), the body burden was calculated assuming an oral absorption of 83%. This value corresponds to the calculated absorption of the γ-stereoisomer in mice (Szabo et al., 2010, 2011a) (See **Section 3.1.1.1**). The steady state body burden is usually estimated in mice and human following the equation:

$$\text{body burden} = (F_{abs} \times \text{dose})/K_{el}$$

Where,

dose = daily dose applied (mg/kg bw per day), NOAEL or chronic human dietary

K_{el} = elimination rate [ln(2)/($T_{1/2}$ in days)] (1/days)

F_{abs} = fraction of the chemical absorbed into the body

For mice, the body burden was not calculated at steady state, but at PND10, i.e. the first day of HBCDDs administration and corresponding to the start of a critical period in the development of the rodent brain (see **Section 3.1.5.1**). Consequently, the CONTAM Panel decided not to apply the elimination rate in the calculation of the mouse body burden.

Taking the LOAEL of 0.9 mg/kg bw per day for spontaneous behaviour and adjusting by the mouse oral bioavailability (83%) of γ-HBCDD (Szabo et al., 2010, 2011a), the body burden in mice at the LOAEL is estimated to be 0.747 mg/kg bw.

The chronic human dietary intake corresponding to the calculated body burden at the LOAEL (0.747 mg/kg bw) in the mice is calculated from the same equation:

$$\text{Chronic human dietary intake} = (\text{body burden} \times K_{el})/F_{abs}$$

In the absence of robust information, the Panel assumed the human absorption of HBCDDs (F_{abs}) to be 100%. Due to the uncertainties in the method to estimate the half-life in Geyer et al. (2004, extended abstract, see **Section 3.1.1.2** and **3.5.4**), the worst-case longest half-life for HBCDDs of 219 days was used (K_{el} = 0.00315 $days^{-1}$). Considering these values, the chronic human dietary intake would be:

$$\text{Chronic human dietary intake} = 0.747 \text{ mg/kg bw} \times 0.00315 \text{ days}^{-1}$$
$$= 2.35 \text{ μg/kg bw per day (or 0.00235 mg/kg bw per day)}$$

3.1.5.3. Derivation of a health-based guidance value or margin of exposure approach

The CONTAM Panel concluded that the derivation of a health-based guidance value is not appropriate due to limitations in the current database on HBCDDs, including in most studies the lack of information on the stereoisomer composition of the HBCDD mixture tested. Repeated dose reproductive toxicity studies, that include a functional observational battery, showed only sporadic effects.

Instead the margin of exposure (MOE) approach was used for the risk characterisation by comparing the calculated chronic human dietary intake leading to a body burden at the LOAEL of 0.9 mg/kg bw in the mouse study of Eriksson et al. (2006) (see **Section 3.1.5.2**) with the estimated dietary exposure reported in **Section 3.3.1**.

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 for HBCDDs is based on a body burden comparison between animals and humans, the potential toxicokinetic differences can be considered as sufficiently covered, and consequently the default uncertainty factor of 4 for interspecies differences in toxicokinetics is not

needed. The Panel recognised that there could be interspecies differences in toxicodynamics for the critical effects observed, and thus applied the default uncertainty factor of 2.5.

Considering the assumption of 100% absorption of HBCDDs in humans, and use of the worst-case maximum half-life estimated in humans of 219 days, the CONTAM Panel concluded that it was not necessary to apply an uncertainty factor to cover individual differences in kinetics. The Panel recognised that there could be differences in the individual susceptibility in the subpopulation of infants and children. Therefore, the Panel concluded that the MOE should also cover individual differences in dynamics (a factor 3.2).

The Panel applied an additional uncertainty factor of 3 to extrapolate from a LOAEL to a NOAEL. This default factor is considered sufficiently conservative (ECHA, 2012; EFSA Scientific Committee, 2012).

The Panel considered whether an additional factor should be applied to allow for limitations in the database. It was noted that reproductive toxicity studies, that include a battery of tests for evaluating potential neurotoxicity, showed only sporadic effects. It was also noted that there is no indication that HBCDDs are carcinogenic (see **Section 3.1.5.1**). Taking all this information into account, it was concluded that an additional uncertainty factor for limitations in the database is not needed.

Thus, the Panel concluded that an MOE higher than **24** (2.5 × 3.2 × 3) would indicate a low health concern.

3.2. Occurrence data

3.2.1. Occurrence data submitted to EFSA

An initial number of 11,770 analytical results (5,122 samples) on HBCDDs in food were available in the EFSA database. Data were reported by 11 European countries.[32] The analytical results were obtained between 2000 and 2018. Because the previous Opinion included data collected up to 2010, and in order to have a data set representing current occurrence levels while retaining a sufficiently high number of samples, the present assessment used data submitted to EFSA between 2010 and 2018.

The occurrence data were carefully evaluated, and a number of validation steps were applied before being used to estimate the dietary exposure (see **Annex B**, Table B.1 for further details). The resulting data set included a total of 7,593 analytical results (2,530 samples) on HBCDDs (α-HBCDD, β-HBCDD, γ-HBCDD and Total HBCDDs) in food.

Data providers were contacted to clarify inconsistencies identified during the data check. The following modifications were made to the initial data set based on the feedback received:

- The product description of several records allowed a more accurate FoodEx classification. In these cases, the samples were reclassified to a more specific, lower level.
- Special attention was given to the analytical method reported. There were samples on Total HBCDDs which were reported being analysed by LC/MS. After clarifying with the data providers, the analytical method was changed to GC/MS. In addition, there were samples on the individual stereoisomers with reported analytical method 'unspecified' but containing some information in the analytical method text. These were clarified further with the data providers and the analytical method was adjusted accordingly. In the final data set, all samples reported on Total HBCDDs were clarified to be analysed by GC/MS and the three major HBCDD stereoisomers by LC/MS.
- 303 occurrence values were reported as 'HBCDDs unspecified'. Since data on the same samples for the three major stereoisomers were also reported, it was decided to exclude them.
- 187 samples data were reported on both Total HBCDDs and individual stereoisomers. Checking these data in detail, it was clarified that the Total HBCDDs reported was not analysed separately but it was the calculated sum of the values provided for the individual stereoisomers. These 187 occurrence values on Total HBCDDs were excluded from the data set.
- Given that α-HBCDD is the predominant stereoisomer in food, three samples that reported only values on β- and γ-stereoisomers, i.e. six occurrence values in total, were excluded from the data set. On that basis, two samples that reported values only on the α- isomer were kept and used for the estimation of exposure to HBCDDs.
- Some occurrence values were expressed on fat weight, but the fat content of the food item was not provided. However, the fat content was required for the calculation of the values on whole weight. For these occurrence values, the median fat content reported for the same food category by other countries was used.

[32] Occurrence data included in the assessment were submitted to EFSA when the UK was a member of the EU.

- A high variability was noticed in fat content reported within certain FoodEx categories. For these entries, as well, the same approach of using the median values was followed. One of the affected food categories was the group of 'Salmon and trout (Salmo spp.)'. Since this is a single category at the most detailed level of the FoodEx with substantially different fat content, it was decided to split it whenever possible to identify the species from the product description. This resulted in having two new food categories: 'Salmon' and Trout'.
- For 141 samples, occurrence data were reported only for Total HBCDDs. The majority of these samples were left-censored (analytical data below LOD or LOQ) with high LOQs. Therefore, these values were excluded from the data set.
- 99 occurrence values were reported as 'suspect sampling' and were excluded.

In the final data set (6,857 occurrence values from 2,287 samples), 67.7% of the data were reported as 'selective sampling', 32.1% as 'objective sampling', while the remaining 0.2% were reported as 'convenient sampling'.[33] It was decided to retain all samples regardless of the sampling strategy.

For all samples in the final data set, values for the three major stereoisomers were available. Therefore, values for the exposure to HBCDDs were calculated as sum of LB/UB of the individual stereoisomers (α-, β- and γ-HBCDD).

As shown in **Figure 2**, occurrence values from the final data set were reported by seven European countries, most of them by France (42.5%) and Germany (35.6%). The majority of the data (79%) were reported between 2012 and 2016 (**Figure 3**).

The left-censored data accounted for 71% of the occurrence values. Both LOQ and LOD were provided for 46% of all left-censored data, while only LOD or only LOQ were provided for 26% and 28% of the reported left-censored occurrence values, respectively. Among those, six food categories were 100% left-censored as shown in **Figure 4**. On the other side, the food group with the highest percentage of quantified data was 'Fish and other seafood (including amphibians, reptiles, snails and insects)'.

Based on the FoodEx classification, 12 food categories at FoodEx Level 1 were represented (**Figure 4**). After the most represented group, 'Fish and other seafood' with 3,554 occurrence values (1,186 samples) reported, the food group with the highest number of samples was 'Meat and meat products (including edible offal)' with 1,656 occurrence values (552 samples). The food groups 'Eggs and egg products' and 'Milk and dairy products' followed with 666 occurrence values (222 samples) and 645 occurrence values (215 samples) reported, respectively.

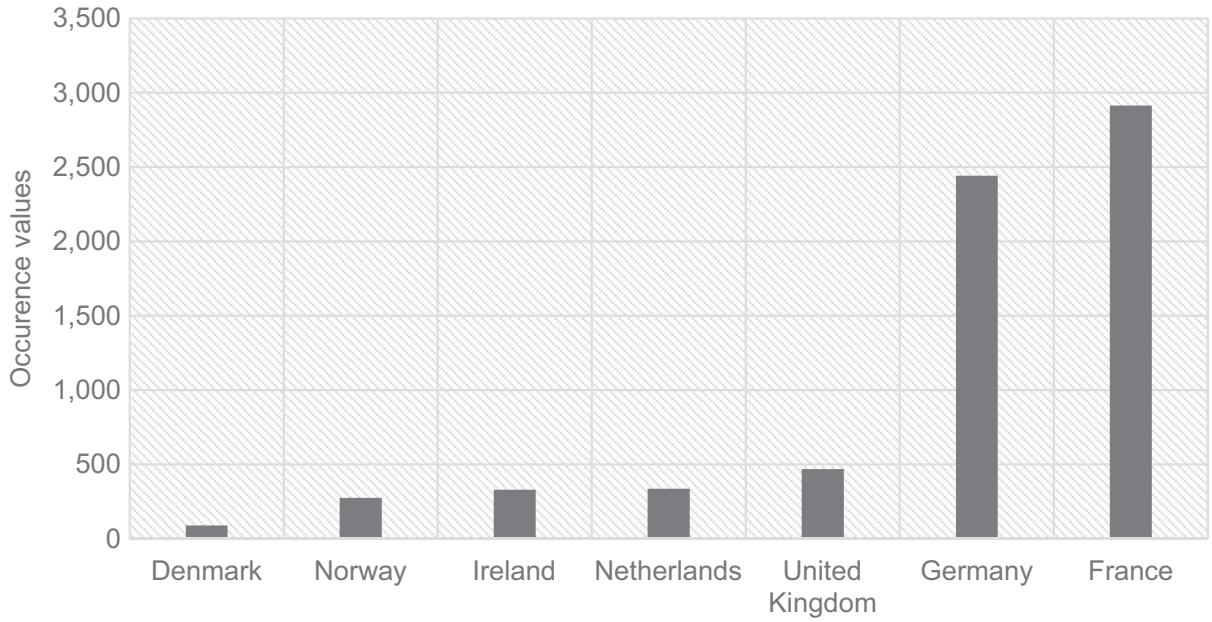

Figure 2: Distribution of occurrence values reported on HBCDDs across different European countries

[33] Definitions on different sampling strategies can be found in the EFSA Guidance on Standard Sample Description (SSD) for Food and Feed (EFSA, 2010a, 2013).

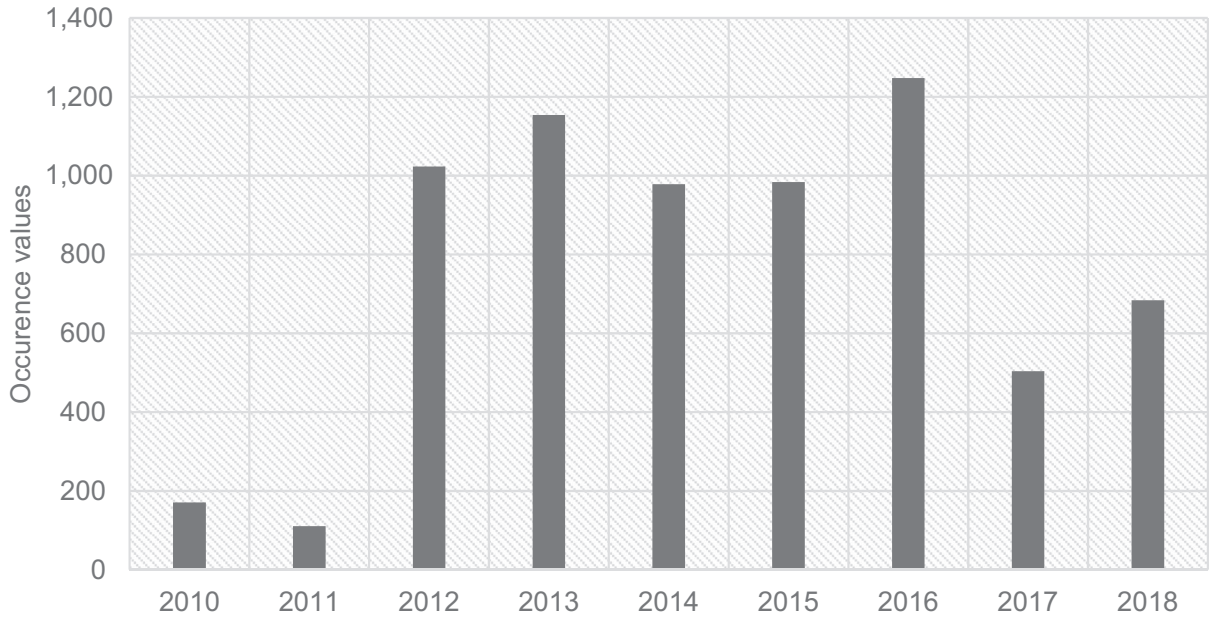

Figure 3: Distribution of occurrence values reported for HBCDDs by year

In view of the exposure assessment, food data were grouped at different FoodEx levels and other merged categories used as supplements to FoodEx, taking into consideration several factors including the similarities between food categories, the number of samples and the concentrations observed.

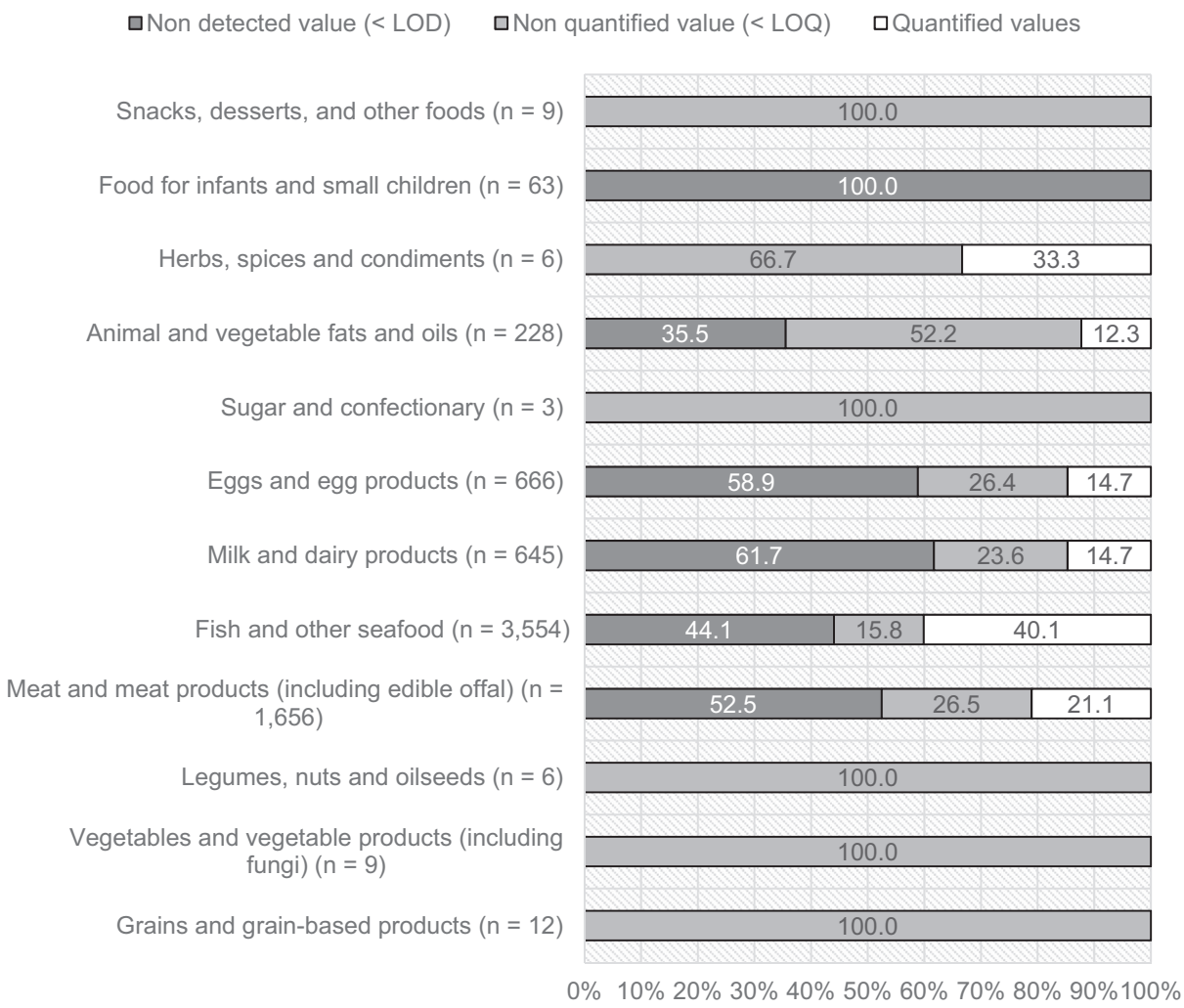

Figure 4: Percentage of analytical results below LOD, below LOQ and quantified values in the final data set across the different food categories (FoodEx Level 1)

At the most detailed level (FoodEx Level 3), the food category was retained if six or more samples were available for that category. If fewer than six samples were available or data for a food category were 100% left-censored, the concentrations were compared with similar foods under the same upper level category:

- If the concentrations were similar, the food was considered at a higher (parent) level (FoodEx Level 2) if this broader category was well-represented by the available categories at FoodEx Level 3. For example, 31 samples of different fish species (i.e. Bream, Carp, Halibut, etc.) represented by fewer than six samples were considered at the parent FoodEx Level 2 'Fish meat'. Similarly, five food categories represented with six or more samples at FoodEx Level 3 but that were 100% left-censored were considered at the parent FoodEx Level 2. This was for example, the case of 'Goose meat (Anser, Branta, Chen)' taken into account at the parent FoodEx Level 2 'Poultry'.
- Categories where the analytical results were 100% left-censored and HBCDDs contamination was not expected, were not taken into account in the assessment. Based on these criteria, 'Snacks, deserts and other foods', 'Herbs, spices and condiments', 'Sugar and confectionary', 'Legumes, nuts and oilseeds', 'Vegetables and vegetable products (including fungi)' and 'Grains and grain-based products' (15 samples in total) were not included in the estimation of exposure to HBCDDs because they were 100% left-censored and/or represented with fewer than six samples.
- All food categories at Level 3 with fewer than six samples and where HBCDDs contamination was expected, were either attributed to Level 2 as described in the first bullet point above or

were excluded if the upper food category was not well represented. This was the case for example, of 'Liver, game animals'. In addition, 13 samples in the Level 2 food category 'Cereals-based food for infants and young children' were also excluded because they were 100% left-censored and were the only samples reported in this higher (parent) level.

Samples in the food categories of the least detailed FoodEx classification (FoodEx Level 1) were excluded if no further information was available for a more specific reclassification. The exception to this was 27 samples reported as 'Meat and meat products (including edible offal)' which were considered at FoodEx Level 2, i.e. as 'Livestock meat'.

Mean occurrence values for the food categories considered at FoodEx Level 3 are calculated as average of all samples reported within the same food category. Samples reported at FoodEx Level 2 were grouped together with associated samples reported at the more detailed level (Level 3) for the purposes of calculating the mean concentrations. For example, mean occurrence values for the samples reported/grouped as 'Fish meat', were calculated using all samples for which Level 2 was 'Fish meat' including those reported at more detailed levels.

For the detailed list of the categories considered see **Annex B** (Table B.2). Overall, 77 food samples (231 occurrence values) from the 2,287 samples (6,857 occurrence values) were not considered for the assessment as they did not fulfil the above selection criteria. In total, 48 food categories were considered for the linking between food occurrence and food consumption data (see **Annex B**, Table B.3).

Occurrence data by food category

Mean values of the LB and UB by food category and FoodEx level, used for the estimation of exposure from 'Fish and other seafood' are presented in **Table 13**. The highest mean concentrations among Fish meat samples were reported for 'Eel' (2.33/2.35 μg/kg ww, LB/UB) and 'Grey mullet (Mugil)' (2.06/2.07 μg/kg ww, LB/UB). Mean concentrations reported for the rest of samples belonging to the food category of 'Fish meat' ranged from 0.00/0.01 μg/kg (LB/UB) to 0.80/0.83 01 μg/kg (LB/UB). Details on the concentrations reported for the food categories of 'Crustaceans', 'Fish offal' and 'Water molluscs' are presented in **Table 13**.

Mean values of the LB and UB for food categories other than 'Fish and other seafood' used for the estimation of exposure are presented in **Table 14**. Mean concentrations for the LB ranged from 0.00 μg/kg ww for 'Butter', 'Pork kidney', 'Game mammals' and 'Cow milk' to 0.53 μg/kg ww for 'Turkey meat (*Meleagris gallopavo*)'. The lowest mean occurrence value for the UB has been reported for 'Game mammals' (0.01 μg/kg ww) while the highest value was reported for 'Turkey meat (*Meleagris gallopavo*)' (0.58 μg/kg ww) (**Table 14**).

More details on grouping of samples within the food category are provided in **Annex B** (Table B.3). In addition, frequency distribution of LB and UB for relevant food categories is presented as violin plots with integrated box plots on the log10 scale (**Annex B**, Figure B.1 and B.2, respectively). The plot related to the LB shows only quantified values.

Table 13: Mean LB and UB values, as used for the exposure assessment, for the food category 'Fish and other seafood'

FoodEx Level 3	FoodEx Level[(a)]	Mean LB[(b)] (μg/kg ww)	Mean UB[(b)] (μg/kg ww)
Crustaceans	2	0.01	0.05
Shrimps (*Crangon crangon*)	3	0.00	0.05
Crab (*Cancer* spp.)	3	0.02	0.07
Fish meat	2	0.19	0.25
Plaice (*Pleuronectes*)	3	0.00	0.11
Sole (*Limanda*; *Solea*)	3	0.01	0.09
Sea catfish and wolf-fish (*Anarhichas*)	3	0.01	0.04
Lophiiformes (*Pediculati*)	3	0.01	0.02
Cod and whiting (*Gadus* spp.)	3	0.02	0.06
Tuna (*Thunnus*)	3	0.02	0.09
Salmon and trout (*Salmo* spp.)	3	0.12	0.17
Sardine and pilchard (*Sardina*)	3	0.13	0.14

FoodEx Level 3	FoodEx Level[(a)]	Mean LB[(b)] (µg/kg ww)	Mean UB[(b)] (µg/kg ww)
Trout	3	0.14	0.20
Hake (*Merluccius*)	3	0.16	0.17
Salmon	3	0.17	0.27
Mackerel (*Scomber*)	3	0.49	0.51
Herring (*Clupea*)	3	0.51	0.54
Bass (*Marone*)	3	0.80	0.83
Grey mullet (*Mugil*)	3	2.06	2.07
Eels	3	2.33	2.35
Fish offal	2	0.93	1.18
Water molluscs	2	0.07	0.07
Scallop (*Pecten* spp.)	3	0.01	0.01
Clam (*Mya arenaria*)	3	0.01	0.01
Queen scallop (*Chlamys opercularis*)	3	0.01	0.01
Oyster (*Ostrea edulis*)	3	0.06	0.07
Mussel (*Mytilus edulis*)	3	0.12	0.12

(a): FoodEx level equal to 2 means that samples were reported/grouped at the second level of the FoodEx; FodEx level equal to 3 means that samples were reported at the third level of the FoodEx.
(b): The values are rounded to two decimals.

Table 14: Mean LB and UB values, as used for the exposure assessment, for food categories other than 'Fish and other seafood'

FoodEx Level 3	FoodEx level[(a)]	Mean LB[(b)] (µg/kg ww)	Mean UB[(b)] (µg/kg ww)
Animal fat	2	0.06	0.46
Tallow	3	0.10	0.57
Pork lard (Schmaltz)	3	0.01	0.46
Chicken fat	3	0.01	0.37
Butter	3	0.00	0.49
Edible offal, farmed animals	2	0.01	0.03
Mutton/lamb liver	3	0.02	0.03
Pork kidney	3	0.00	0.03
Eggs, fresh	2	0.17	0.20
Whole egg, chicken	3	0.16	0.19
Game mammals	2	0.00	0.01
Liquid milk	2	0.01	0.03
Cow milk	3	0.00	0.03
Livestock meat	2	0.03	0.05
Beef meat (*Bos* spp.)	3	0.05	0.08
Pork/piglet meat (*Sus scrofa*)	3	0.04	0.05
Mutton/lamb meat (*Ovis aries*)	3	0.02	0.04
Rabbit meat (*Lepus cuniculus*)	3	0.01	0.03
Poultry	2	0.18	0.21
Turkey meat (*Meleagris gallopavo*)	3	0.53	0.58
Chicken meat (*Gallus domesticus*)	3	0.01	0.03

(a): FodEx level equal to 2 means that samples were reported/grouped at the second level of the FoodEx; FodEx level equal to 3 means that samples were reported at the third level of the FoodEx.
(b): The values are rounded to two decimals.

3.2.2. Previously reported occurrence data

The previous EFSA Opinion on HBCDDs (EFSA CONTAM Panel, 2011a) summarised the occurrence data on HBCDDs in food submitted by European countries as well as those published in peer-reviewed journals. Fish and other seafood were the food groups with the highest number of samples.

Depending on the fishing ground, the HBCDD levels covered a quite broad contamination range. The occurrence data in other food groups were characterised by a high proportion of non-detects. Generally, α-HBCDD was the predominant stereoisomer found in food of animal origin. Exceptions were, e.g. fish samples caught in rivers downstream of highly industrialised areas which showed higher concentrations for γ-HBCDD, the major stereoisomer in technical HBCDD.

The following paragraphs, which do not claim for completeness, give a short summary on HBCDDs occurrence in food collected in Europe and published since the previous EFSA Opinion on HBCDDs. The primary focus of the recently published occurrence data was on fish and marine sea food. As already indicated in the previous Opinion, α-HBCDD prevailed in most studies and the median levels, especially in fish caught for consumption was generally below 1 μg/kg ww (Munschy et al., 2013, 2015; Kuc et al., 2014; Aznar-Alemany et al., 2017; Fliedner et al., 2018; Malysheva et al., 2018; Nøstbakken et al., 2018; Vénisseau et al., 2018). However, a number of samples were detected where γ-HBCDD was the dominating stereoisomer. The reason for this, whether due to point sources or eventually influenced by the species, is not known. In addition, a number of substantially higher contamination levels were found, especially in mackerel and mussels. In general, the contamination levels in samples from the Mediterranean were higher than from the North Sea, the English Chanel and the North Atlantic.

The HBCDD contamination of 25 fish oils for feed and food of different origin was assessed by Ortiz et al. (2011). Total HBCDDs ranged from 0.09 to 26.8 μg/kg, with higher concentrations in fish oil for feed (average of 9.69 μg/kg) than those for food (1.14 μg/kg). In general, fish oils from Northern Atlantic presented higher levels (4.78–15.49 μg/kg) than those from the Southern Pacific (3.34–5.49 μg/kg).

Vénisseau et al. (2018) studied the occurrence of legacy and novel brominated flame retardants, including HBCDDs in more than 600 food and feed samples from France collected for the period 2014–2016 in the context of French monitoring plans. The broadest contamination range for the prevailing α-HBCDD stereoisomer was found in fish, fish-derived products, such as fish meal and fish oil for feed and shellfish. The range for α-HBCDD in fish meal (n = 15), fish oil for feed (n = 15), fish (n = 114) and crustaceous/molluscs (n = 154) was determined as < LOD–1.08 (median: 0.024), 0.061–2.1 (median: 0.697), < LOD–3.29 (median: 0.0235) and < LOD–0.368 (median: 0.021) μg/kg ww, respectively. While the frequency of detection in these food and feed commodities was between 67 and 100%, the respective ranges for β-HBCDD and γ-HBCDD were between 24–40 and 33–87%, respectively. Compared to fish, fish derived products and marine sea food, the range of HBCDD levels in food of animal origin was substantially smaller. The concentration ranges for α-HBCDD in milk (n = 72), eggs (n = 57), sheep liver (n = 28) and meat (n = 152) were reported as < LOD–0.00987 (median: < LOD), < LOD–0.4152 (median: 0.00204), < LOD–0.1337 (median: < LOD) and < LOD–0.2325 (median: 0.00118) μg/kg ww, respectively. The frequency of detection ranged between 21 and 54% and thus is lower compared to fish, fish derived products and shellfish. For β-HBCDD and γ-HBCDD the frequency of detection in the food samples of animal origin ranged between 1 and 32%.

Fernandes et al. (2016) measured HBCDDs in most commonly consumed foods (n = 156) and animal feeds (n = 51) sampled in the UK. The food samples comprised fish and shellfish, milk, dairy products, eggs, meat, processed meat, offal, processed and 'other' foods. Fish, shellfish and processed meats showed the most frequent detections and also the highest HBCDD concentrations, where α-HBCDD was predominant. The ranges for HBCDDs in the various food groups were generally between < 0.01 and 0.1 μg/kg ww with higher levers found in fish and shellfish where maximum concentrations for α-HBCDD of 10.13 and 3.41 μg/kg ww were determined.

García López et al. (2018) assessed the HBCDD contamination of 53 composite food samples from Ireland collected between July and December 2015. The composite samples contained several hundred subsamples of eggs, milk, fish, fat (ovine, bovine, porcine and avian fat) and liver. Fish showed the broadest contamination range for the sum of HBCDDs (0.07–0.41 μg/kg ww) with a mean value of 0.28 μg/kg ww. A somewhat lower mean value of 0.23 μg/kg ww was found for fat (range: 0.06–0.20 μg/kg ww). Substantially lower HBCDD levels were found in egg yolk and liver with mean values of 0.01 and 0.05 μg/kg ww, respectively. In milk samples, HBCDDs were not detected above 0.01 μg/kg ww.

Poma et al. (2018) reported on the occurrence of HBCDDs and other flame retardants in a total of 183 composite food samples collected in Belgium. Mean levels for the sum of the three HBCDD stereoisomers in fish, meat and meat products, milk and milk products and eggs and egg products were 0.131, 0.043, 0.0001 and 0.971 μg/kg ww, respectively. The high mean value in eggs and egg products was mainly attributed to the presence of α-HBCDD (3.885 μg/kg ww) in one organic egg

sample. In food for infants and small children, animal and vegetable fat, grains and grain products and potatoes and derived products, HBCDDs could not be detected.

HBCDD levels in chicken eggs are generally low. Nevertheless, in a few cases concentrations of more than 3,000 μg/kg lipid were reported. As a plausible reason, Jondreville et al. (2017b) have demonstrated that hens can ingest HBCDD-containing extruded polystyrene (XPS) which is occasionally used as insulating material in rearing buildings. Due to the addition of HBCDDs to XPS which can be in the percent range, ingestion of this material by hens can lead to extraordinary HBCDD levels in chicken eggs.

Dervilly-Pinel et al. (2017) assessed the chemical contamination levels of both conventional and organic meats. Besides α-, β- and γ-HBCDD the study included a number of other inorganic and organic environmental contaminants together with chemical residues arising from production inputs. The type of farming investigated was conventional standard, Label Rouge and organic farming. Label Rouge which is a French national quality assurance scheme for meat production (e.g. prescribing outdoor access, age and weight at slaughter) is closer to organic than to standard meat production. Median HBCDD contamination levels (sum of three stereoisomers) in bovine from conventional (n = 42) and organic farming (n = 43) were 0.03 and 0.04 μg/kg lipid, respectively. Discrimination between the two farming practices was not possible. Regarding porcine meat, the median value for the sum of the three stereoisomers in samples from conventional standard farming (n = 41) being 0.11 μg/kg lipid was lower compared to products from Label Rouge farming (n = 12) and organic farming (n = 43) with medians of 0.16 μg/kg lipid and 0.30 μg/kg lipid, respectively. The highest concentration of 194.8 μg/kg lipid was determined in a sample from conventional standard farming. Analyses of broiler meat for the three stereoisomers revealed a median of 0.17 μg/kg lipid for samples from conventional standard farming (n = 31) which was significantly lower ($p < 0.001$) than the medians for meat samples from Label Rouge farming (n = 13) and organic farming (n = 41) with medians of 0.34 and 0.33 μg/kg lipid, respectively.

In a monitoring study conducted in France from 2013 to 2015, Huneau-Salaun et al. (2020) measured the levels of HBCDDs in 60 hen egg farms (34 without an open-air range and 26 free-range), 57 broiler farms (27 without an open-air range and 30 free-range) and 42 pig farms in relation to their rearing environments. The authors measured the levels of HBCDDs in eggs, broiler muscle and pig muscle and found that α-HBCDD was the most frequently detected stereoisomer found in eggs, broiler muscle and pig muscle, while β-HBCDD was reported in broilers muscles, and γ-HBCDD in broiler and pig muscle. The authors concluded that the contamination of free-range eggs and broilers was more frequent than that of conventional ones, suggesting that access to an open-air range could be an additional source of exposure to HBCDDs. The authors could not establish any direct relationship between the occurrence of HBCDDs in eggs and meat and the characteristics of farm buildings (such as age, building materials).

The above data from European countries show a broad range of HBCDD concentrations depending on the food commodity and its origin of sampling. This is also true for samples from non-European countries. A number of studies were conducted especially in China (Xia et al., 2011; Meng et al., 2012; Qiu et al., 2012; Zhu et al., 2013; Yin et al., 2015; Shi et al., 2017), the US (Schecter et al., 2010, 2012), Canada (Su et al., 2018) and Japan (Ueno et al., 2010; Kakimoto et al., 2012), which confirm the ubiquitous distribution of HBCDDs and their broad contamination of food.

A compilation of global occurrence levels on HBCDDs and other flame retardants in food is presented in the recent publication by Aznar-Alemany and Eljarrat (2020).

The impact of e-waste recycling plants on the HBCDD concentrations in eggs from free ranging chicken were demonstrated in several papers (Zheng et al., 2012; Tao et al., 2016b; Zeng et al., 2016; Huang et al., 2018). Levels of several thousand μg/kg fat were reported resulting in considerable dietary exposure when consumed by the inhabitants living in these areas.

The Panel noted that the data that were submitted by European countries on HBCDD concentrations in food from official food control are in a comparable range as the results published during the last decade in the peer-reviewed literature. In general, the HBCDD concentrations in food for human consumption indicate to levels below 1 μg/kg ww. However, literature data also showed some substantially higher HBCDD concentrations in several specimens depending on the origin of sampling, such as in samples collected in contaminated areas, or in eggs and egg products from hens that were housed in rearing buildings where HBCDD containing extruded polystyrene (XPS) was used as insulating material.

3.2.3. Food processing

Data on effects of food processing on the HBCDD concentrations in food are scarce. Regarding food of animal origin, and considering that HBCDDs are lipophilic compounds, it can be assumed that all processing steps which alter the lipid content of a food commodity, such as trimming of visible fatty tissue will have an impact on the HBCDD levels in the processed sample. As HBCDDs are relatively stable compounds degradation at typical temperatures during food and feed processing seem unlikely. However, the γ-HBCDD stereoisomer can be rearranged to form α-HBCDD at temperatures of 190°C (Peled et al., 1995) (see **Section 1.3.1**).

The effect of steaming at 105°C for 15 min for fish, and at 105°C for 5 min for bivalves, each wrapped in aluminium foil, on the levels of various contaminants was investigated by Alves et al. (2017). In the case of α-HBCDD, concentrations were similar in raw and steamed plaice and mussel, respectively ($p > 0.05$).

Aznar-Alemany et al. (2017) analysed 14 fish samples both raw and steamed with no further details on steaming conditions given. Total HBCDD levels seemed to behave differently depending on the species with increased levels for mackerel, mixed results for mussel and loss of contamination for plaice, tuna, salmon and sea bream.

Rasmussen et al. (2017) investigated the effect of industrial cold smoking on various contaminant levels, including α-, β- and γ-HBCDD, in Greenland halibut and farmed Atlantic salmon. The mean α-HBCDD concentration in the raw and processed Greenland halibut fillets (0.06 vs. 0.05 μg/kg ww) and the farmed Atlantic salmon (0.18 vs. 0.20 μg/kg ww) were not significantly different ($p > 0.05$). β- and γ-HBCDD could not be detected at an LOD of 0.05 μg/kg ww.

Zhang et al. (2017b) systematically studied the changes of HBCDDs during the refining process, including neutralisation, bleaching and deodorisation in peanut, corn and pressed and extracted soybean oils. The concentrations of HBCDDs in the crude oils of peanut, corn, pressed soybean and extracted soybean oils were 0.04, 0.06, 0.52 and 0.10 μg/kg, respectively. In all oils α-HBCDD was by far the predominant stereoisomer. During the refining process, the mean concentrations of α-HBCDD detected in peanut, corn and extracted soybean oils were reduced by 25%, 17% and 100%, respectively. In contrast to the above oils, all three diastereoisomers were detected in all the pressed soybean oil samples. While α- and β-HBCDD were reduced during the refining process by 90% and 82%, respectively, there was no significant reduction of the γ-HBCDD levels.

Several authors studied the presence of HBCDDs in kitchen hoods and utensils for food preparation. Bendig et al. (2013) analysed fat residue from 15 kitchen hoods for several polyhalogenated compounds. Total HBCDD was detected in three samples at 8.8–11 μg/kg fat. Additionally, one sample contained the HBCDD metabolite pentabromocyclododecene at 350 μg/kg fat, which was about 40 times higher than HBCDDs. The results indicate that kitchen hood fat may serve as a valuable alternative matrix for the assessment of indoor contaminations with polyhalogenated compounds.

Samsonek and Puype (2013) investigated brominated flame retardants in black thermo cups and selected kitchen utensils purchased on the European market. They did not find HBCDDs in any of the products tested using X-ray fluorescence spectrometry (XRF) to screen products for total bromine followed by thermal desorption GC-MS. Li et al. (2019b) also applied portable XRF combined with chemical analysis to investigate the occurrence, profile and distribution of the most important BFRs (PBDEs, HBCDDs and TBBPA) in 120 consumer products on the Chinese market, with the aim of identifying the possible reservoirs of persistent BFRs in products in China. Of these, 30 were daily-use household items, including storage boxes, fruit bowls and cups and bottles. Bromine was only detected in four daily-use household products within a low concentration range.

In summary, the data on the fate of HBCDDs during food processing are scarce. Although one report showed degradation of HBCDDs in kitchen hoods, due to their thermal stability it seems unlikely that they are formed or degraded during temperatures typical for food processing. Thus, potential reduction of their concentration in processed foods may be mainly caused by loss of fat, rather than degradation. Nevertheless, the γ-HBCDD stereoisomer can be rearranged to form α-HBCDD at 190°C resulting in a different stereoisomeric pattern during food processing. It cannot be excluded that contact with BFR containing kitchen consumer goods may lead to contamination of the respective food due to migration.

3.3. Dietary exposure assessment

3.3.1. Current dietary exposure assessment

The CONTAM Panel assessed the dietary exposure to HBCCDs (sum of α-, β- and γ-HBCDD) following the methodology described in **Section 2.5**. A summary of the HBCDD occurrence data including the number of results and mean concentrations across the FoodEx level food categories as used for exposure assessment in presented in **Section 3.2.1**.

Mean and high dietary exposure

The mean and 95th percentile chronic dietary exposure to HBCDDs (ng/kg bw per day) was estimated separately for each consumption survey using data recorded at the individual level from the Comprehensive Database (see **Section 2.3**). Due the methodological differences among the surveys, chronic dietary exposure was estimated separately for each of them.

It was not possible to assess the dietary exposure to HBCDDs for 'Infants' using the data submitted to EFSA through the call for data since the occurrence data available in the food group 'Food for infants and small children'[34] was limited (only 19 samples) and was all left-censored. Exposure of breastfed infants to HBCDDs was assessed using data from a global WHO/UNEP study (see **Section 3.1.1.4**).

Table 15 shows the summary statistics for the assessment of chronic dietary exposure to HBCDDs. Detailed mean and 95th percentile dietary exposure estimates calculated for all population groups for each of the 44 dietary surveys are presented in **Annex D** (Tables D.1 and D.2). The total dietary intake was estimated using the LB and UB HBCDD concentrations from the calculated sum of α-, β- and γ-HBCCD for all selected food groups.

Table 15: Summary statistics for the chronic dietary exposure to HBCDDs (sum of α-, β- and γ-HBCDD) (ng/kg bw per day) across European countries

Age group	Minimum		Median		Maximum	
	LB	UB	LB	UB	LB	UB
	Mean dietary exposure in total population (ng/kg bw per day)[(b)]					
Toddlers	0.14	0.63	0.33	0.98	0.79	1.52
Other children	0.11	0.37	0.28	0.77	0.63	1.21
Adolescents	0.07	0.19	0.17	0.36	0.34	0.64
Adults	0.08	0.17	0.16	0.29	0.28	0.44
Elderly	0.10	0.19	0.16	0.30	0.23	0.40
Very elderly	0.07	0.19	0.15	0.30	0.30	0.49
	P95 dietary exposure in total population (ng/kg bw per day)[(b)]					
Toddlers[(a)]	0.58	1.54	0.90	2.16	2.30	3.61
Other children	0.37	1.01	0.78	1.66	2.05	2.95
Adolescents[(a)]	0.23	0.51	0.44	0.86	1.20	1.54
Adults	0.29	0.48	0.46	0.73	1.18	1.37
Elderly	0.30	0.50	0.49	0.74	0.74	0.90
Very elderly[(a)]	0.30	0.45	0.44	0.74	0.78	0.97

bw: body weight; LB: lower bound; UB: upper bound.
(a): The 95th percentile estimates obtained on dietary surveys/age groups with fewer than 60 observations may not be statistically robust (EFSA, 2011a) and are therefore not included in this table.
(b): The values are rounded to two decimals.

The highest estimated chronic dietary exposure to HBCDDs (except for breastfed infants) was in the younger age groups, in particular in 'Toddlers'. For this age group, the mean dietary estimates range from 0.14 to 1.52 ng/kg bw per day (minimum LB and maximum UB, respectively). The 95th percentile exposure ranges from 0.58 to 3.61 ng/kg bw per day (minimum LB and maximum UB, respectively).

[34] Description as in FoodEx.

Dietary exposure in specific groups of the population, namely 'Pregnant women' and 'Lactating women', were within the range of exposure estimates for the adult population (see **Annex D**, Tables D.1 and D.2).

In the previous Opinion (EFSA CONTAM Panel, 2011a), due to lack of consumption data, an exposure estimation for toddlers was not possible which precludes a comparison with the current assessment. The age group 'Other children' was identified in the former Opinion to have the highest dietary exposure to HBCDDs. The mean and P95 dietary exposure then ranged between 0.15–1.85 and 0.80–4.46 ng/kg bw per day, respectively. In the current assessment, the respective estimated dietary exposure is somewhat lower, being 0.11–1.21 and 0.37–2.95 ng/kg bw per day, for mean and P95 dietary exposure, respectively. The same holds true for dietary HBCDD exposure for adults. While the mean and P95 dietary exposure across dietary surveys in European countries in the former HBCDD Opinion was estimated as 0.09–0.99 and 0.39–2.07 ng/kg bw per day, respectively, the current estimates range between 0.08–0.44 and 0.29–1.37 ng/kg bw per day, for mean and P95 dietary exposure, respectively.

In the previous Opinion (EFSA CONTAM Panel, 2011a), a scenario for high and frequent fish consumers was estimated as high consumption of fish meat was considered as a special diet with specific concern for dietary exposure to HBCDDs. In the current Opinion the intake estimations of HBCDDs for high and frequent fish meat and fish offals consumers are represented by the 95th percentile reported in **Table 15**.

It can be assumed that the dietary HBCDD exposure for vegetarians is lower than that for people consuming a mixed diet. This is because 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.

Contribution of different food categories to the chronic dietary exposure

The contribution of individual food categories to the LB and UB mean chronic dietary exposure to HBCDDs varied between the dietary surveys and is shown in **Annex D** (Tables D.4 and D.5). This is explained by the specific food consumption patterns in the individual European countries and even in different regions within a country. The detailed contribution to the mean LB chronic dietary exposure to HBCDDs of the different food categories at FoodEx Level 2 and grouped by age class, country and survey as used for the exposure assessment and grouped by age classes is shown in **Annex D** (Table D.3).

In **Table 16,** the relative contribution (%) of each food category to the overall mean LB and UB exposure of HBCDDs as median and range (minimum and maximum) for each age class across all European dietary surveys is presented. Dietary exposure reflects the pattern of consumption figures of each age class and the respective country as well as occurrence values.

When a high proportion of left-censored data produce a large difference between UB and LB occurrence values, this results in a commensurate uncertainty in dietary exposure estimates. Appraising the contribution of the respective food groups to the total LB exposure is based on measured values not influenced by the percentage and magnitude of the left-censored data. Appraising the contribution of food groups to the total UB dietary exposure should be done with care as the high contribution of certain food groups can be the result of high LOQs or high consumption rather than an actual high contribution.

Four main food categories ('Fish meat', 'Eggs fresh', 'Livestock meat' and 'Poultry') are the main contributors for the LB estimates. Six food categories ('Fish meat', 'Eggs fresh', 'Livestock meat', 'Poultry', 'Liquid milk' and 'Animal fat') are the main contributors for the UB estimates (highlighted in grey in the table).

Considering the LB estimates, which are less influenced by the values of the LOD/LOQ, the contribution of 'Fish meat' to the median intake of HBCDDs across European dietary surveys varies from 22% in 'Adolescents' to 47% in 'Elderly'. A considerably lower contribution to the dietary intake of HBCDDs is observed when the UB estimates are considered, where the contribution of 'Fish meat' to the median intake of HBCDDs varies from 11% in 'Toddlers' to 30% in 'Elderly'.

The food category of 'Eggs, fresh' is also significantly contributing to the dietary intake of HBCDDs with a median LB contribution across European dietary surveys ranging from 18% in 'Very elderly' to 27% in 'Other children'. The median UB contribution is lower, ranging from 8% in 'Toddlers' to 13% 'Other children'.

For the food group 'Livestock meat', the median LB contribution ranges from 14% in 'Elderly' to 21% in 'Adolescents', whereas considering the median UB, the contribution ranges from 7% in 'Toddlers' to 13% in 'Adults'.

The food category 'Poultry' is also contributing to the dietary intake of HBCDDs with a median LB contribution across European dietary surveys ranging from 16% in 'Adolescents' to 19% in 'Very elderly', whereas considering the median UB, the contribution ranges from 9% in 'Toddlers' to 14% in 'Adults'.

For the food group 'Liquid milk', the median LB contribution ranges from 1% in 'Elderly' to 9% in 'Toddlers'. The median UB contribution is much higher ranging from 14% in 'Elderly' to 44% in 'Toddlers'.

The category of 'Animal fat' is only contributing in the UB estimates with values ranging from 8% in 'Toddlers' to 17% in 'Very elderly'.

The distinct influence of left-censored data on the calculation of the distribution of different food categories to the chronic dietary exposure can best be demonstrated by the contribution of 'Liquid milk'. While the LB contribution ranges from 1.3–8.7% across the different age classes, the corresponding UB values amount to 14.3–42.8%. As a result, the contribution of other food groups decreased.

In the previous HBCDD Opinion (EFSA CONTAM Panel, 2011a), the contribution of 'Fish meat and products' to the median LB intake of HBCDDs across European dietary surveys varied from 83% to 88.2% for the different age groups. In the current assessment, the contribution of fish meat to the LB chronic dietary exposure only ranges between 22% and 47.1%. The CONTAM Panel notes that a comparison of the current data with results from the previous Opinion is hampered by a number of facts, such as improvements in instrumental analysis, different percentage of left-censored data, consideration of further food commodities and use of more detailed consumption data.

Table 16: Relative contribution (%) to the overall mean dietary intake of HBCDDs of the food categories across different age classes of the general population

Food category	Age class											
	Toddlers		Other children		Adolescents		Adults		Elderly		Very elderly	
	Median	(Min–Max)	Median	(Min–Max)	Median	(Min–Max)	Median	(Min–Max)	Median	(Min–Max)	Median	(Min–Max)
	LB (%)[(a)]											
Animal fat	0.04	(0.00–0.15)	0.04	(0.00–0.35)	0.03	(0.00–0.57)	0.04	(0.00–1.58)	0.05	(0.00–1.80)	0.05	(0.00–3.61)
Crustaceans	0.04	(0.00–0.18)	0.04	(0.00–0.19)	0.14	(0.01–0.31)	0.08	(0.00–0.51)	0.09	(0.01–0.43)	0.13	(0.02–0.22)
Edible offal, farmed animals	0.06	(0.01–0.63)	0.10	(0.00–0.64)	0.08	(0.01–0.68)	0.13	(0.03–1.11)	0.20	(0.09–0.86)	0.19	(0.04–2.23)
Eggs, fresh	24.09	(9.06–56.38)	27.15	(10.60–43.55)	20.52	(5.52–45.70)	18.77	(5.78–37.34)	17.72	(4.49–37.19)	16.63	(8.95–37.64)
Fish meat	22.90	(12.37–50.01)	25.70	(13.48–45.70)	22.36	(12.19–51.75)	33.54	(9.06–68.71)	47.11	(6.46–65.45)	44.41	(5.03–66.20)
Fish offal	0.24	(0.03–25.81)	0.30	(0.02–9.70)	0.36	(0.04–5.50)	0.50	(0.08–13.67)	1.01	(0.04–7.48)	0.71	(0.02–9.90)
Game mammals	0.01	(0.00–0.08)	0.01	(0.00–0.07)	0.01	(0.00–0.02)	0.01	(0.00–0.08)	0.01	(0.00–0.08)	0.01	(0.00–0.04)
Liquid milk	8.73	(1.77–28.09)	4.91	(1.43–12.80)	3.36	(1.26–12.26)	1.44	(0.60–5.23)	1.30	(0.53–2.90)	1.36	(0.74–3.95)
Livestock meat	15.45	(6.25–31.77)	18.34	(7.30–36.47)	21.18	(10.64–48.17)	18.47	(9.95–34.03)	13.86	(7.38–31.57)	18.28	(7.07–32.36)
Poultry	18.54	(1.85–39.81)	17.32	(4.50–41.62)	16.10	(2.57–55.21)	18.31	(4.06–40.44)	15.84	(3.26–35.84)	19.22	(1.78–29.37)
Water molluscs	0.26	(0.01–1.10)	0.38	(0.00–4.99)	0.51	(0.01–8.15)	0.61	(0.00–4.89)	1.31	(0.04–3.81)	0.37	(0.02–2.59)
	UB (%)[(a)]											
Animal fat	7.85	(0.63–31.48)	9.09	(0.59–36.13)	9.65	(1.37–41.14)	10.43	(1.94–38.93)	15.81	(0.60–32.71)	16.80	(5.44–36.44)
Crustaceans	0.07	(0.01–0.36)	0.12	(0.00–0.58)	0.31	(0.02–1.09)	0.29	(0.00–1.30)	0.30	(0.07–0.88)	0.33	(0.07–0.56)
Edible offal, farmed animals	0.07	(0.01–0.41)	0.09	(0.00–0.55)	0.10	(0.00–0.59)	0.15	(0.05–1.36)	0.25	(0.12–1.02)	0.27	(0.07–2.34)
Eggs, fresh	8.23	(4.10–33.79)	13.28	(4.67–21.10)	9.16	(2.96–21.66)	11.40	(4.87–24.14)	10.17	(3.31–22.18)	9.23	(5.87–22.57)
Fish meat	11.04	(5.09–34.53)	12.56	(6.17–30.19)	13.33	(6.88–29.87)	24.90	(6.56–53.70)	29.50	(4.58–53.18)	27.77	(3.31–53.32)
Fish offal	0.09	(0.02–8.62)	0.12	(0.01–4.39)	0.21	(0.03–2.91)	0.31	(0.06–10.00)	0.80	(0.04–5.88)	0.46	(0.01–7.41)
Game mammals	0.01	(0.00–0.12)	0.02	(0.00–0.10)	0.01	(0.00–0.04)	0.02	(0.00–0.18)	0.03	(0.00–0.21)	0.03	(0.00–0.12)
Liquid milk	44.26	(15.02–70.64)	37.06	(13.38–68.16)	30.31	(13.30–67.59)	15.18	(6.22–30.69)	14.30	(6.12–25.48)	17.80	(7.67–27.48)
Livestock meat	7.80	(3.11–12.72)	8.24	(4.31–14.58)	12.43	(6.48–24.52)	12.66	(7.93–21.92)	11.32	(6.37–22.55)	12.44	(5.77–21.68)
Poultry	9.43	(2.58–22.21)	10.81	(3.68–26.49)	10.67	(4.36–34.78)	14.31	(5.66–33.59)	12.54	(4.53–25.84)	13.74	(2.64–22.32)
Water molluscs	0.11	(0.00–0.63)	0.12	(0.00–2.64)	0.24	(0.00–4.86)	0.33	(0.00–3.07)	0.94	(0.02–2.36)	0.20	(0.01–1.46)

LB: lower bound; UB: upper bound.Note: The median and the range (minimum and maximum) lower (LB) and upper bound (UB) contributions of each food category 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 categories. The values are rounded to two decimals.

(a): Due to the high proportion on non-detects across the individual stereoisomers, the calculation of the UB sum might be overestimated.

Breastfed infants

For the exposure assessment of breastfed 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% (EFSA CONTAM Panel, 2011a).

The exposure scenario based on average human milk consumption and the reported UB range for ΣHBCDD (predominantly α-HBCDD) in pooled human milk samples collected in European countries between 2014 and 2016 as part of the WHO/UNEP field studies (see **Table 8**), would result in a median daily exposure of 14.3 ng/kg bw (range: 3.2–73.7 ng/kg bw). For infants with high human milk consumption the respective median daily exposure would result in 21.5 ng/kg bw (range 4.8–110.6 ng/kg bw).

Using the LB data, the median daily exposure would result in 13.8 ng/kg bw (range: 2.3–73.4 ng/kg bw). For infants with high human milk consumption the respective LB median daily exposure would result in 20.7 ng/kg bw (range 3.4–110.2 ng/kg bw).

The Panel noted that since these were pooled samples, it was not possible to estimate specific values for individuals.

Compared to the previous Opinion on HBCDDs (EFSA CONTAM Panel, 2011a) where daily exposures to HBCDDs from average and high milk consumption of 0.60–142 and 0.90–213 ng/kg bw, respectively, were calculated, the respective estimations in the current assessment, in particular at the upper end, are substantially lower.

3.3.2. Previously reported dietary exposure assessment

In 2011, the CONTAM Panel summarised the occurrence data submitted to EFSA by European countries and assessed chronic dietary exposure based on the concentration of Total HBCDDs 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' (EFSA CONTAM Panel, 2011a). The highest mean estimated dietary exposure to HBCDDs across the European dietary surveys was for children from three to ten years old ('Other children') and was between 0.15 to 1.85 ng/kg bw per day. The range reflects the minimum LB and the maximum UB exposure, respectively. Total dietary exposure for adults was around half the exposure for 'Other children', with minimum LB and maximum UB of respectively 0.09 and 0.99 ng/kg bw per day. For high consumers (95th percentiles), the dietary intake of HBCDDs across European countries for 'other children' were between 0.80 and 4.46 ng/kg bw per day (minimum LB and maximum UB, respectively). The corresponding data for 'adults' were between 0.39 and 2.07 ng/kg bw per day, respectively.

As contamination of food samples of plant origin is generally lower than that of food samples of animal origin, the CONTAM Panel 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 HBCDDs resulted in daily exposures of about 0.6–142 ng/kg bw. For infants with high human milk consumption (1,200 mL per day) the respective daily exposures ranged from 0.90 to 213 ng/kg bw (EFSA CONTAM Panel, 2011a).

The following, which does not claim for completeness, gives a short summary on assessments on dietary exposure to HBCDDs in Europe published in open literature since the previous EFSA Opinion on HBCDDs.

Tao et al. (2017) collected samples of 14 different food groups (three samples each) from two supermarkets representing national chains and one local market in Birmingham, UK during May and June 2015. Besides a number of emerging flame retardants, the study also included HBCDDs. Dietary intakes of the flame retardants were calculated for UK toddlers and adults based on the analytical results of the samples and the food consumption data from the latest national diet and nutrition survey report. Body weight values were assumed to be 70 kg for adults and 10 kg for toddlers, respectively. The estimated average dietary intakes of the sum of α-, β- and γ-HBCDD for UK adults and toddlers are reported to be 8.8 and 31 ng/day, respectively.

Based on the analysis of 42 commercial sea food samples (10 species) and the respective seafood consumption pattern, Aznar-Alemany et al. (2017) estimated a mean daily intake via fish of 0.49 ng/kg bw for Spanish adults. The P99 (high seafood consumers) exposure for the UB scenario resulted in a mean daily intake of 1.1 ng/kg bw.

Coelho et al. (2016a) measured a number of persistent organic pollutants including HBCDD in 7-day duplicate diet samples from 21 Portuguese volunteers. Estimated daily intakes (EDIs) of the target compounds were calculated considering that a person ingests daily 1,867.2 g of food. For HBCDDs, the levels of α-, β- and γ-HBCDD were mostly below LOD, with α-HBCDD being the most frequent isomer detected (23.8%). The estimated daily mean LB and UB intake for the sum of the three HBCDD isomers was reported as 2.0 ng/kg bw (range: 0–37, median: 0 ng/kg bw) and 2.5 ng/kg bw (range: 0.37–37, median: 0.70 ng/kg bw).

Sahlström et al. (2015b) determined the concentrations of HBCDDs among other brominated flame retardants in diet and house dust for a mother-toddler cohort and estimated exposure via these two media. Market basket samples comprised the food categories fish, meat, vegetable oils, dairy products and eggs (4 homogenates for each category) collected in 2010. The median daily intake of the sum of α-, β- and γ-HBCDD from diet for Swedish mothers and toddlers (n = 20) was estimated as 11 ng per day (range: 2.7–21 ng/day) for mothers and 5 ng per day (range: 1.3–9.7 ng per day) for toddlers, respectively.

Rivière et al. (2014) reported on the results of the total diet study (TDS) performed in France 2007–2009. The TDS covered the most important foods in terms of consumption, selected nutrients and contribution to contamination. The mean daily dietary exposure to the sum of α-, β- and γ-HBCDD was 0.211 ng/kg bw and 0.32 ng/kg bw for adults and children, respectively. The P95 was estimated at 0.448 ng/kg bw per day and 0.734 ng/kg bw per day for adults and children, respectively.

Based on consumption data statistics, food items from six food groups, i.e. fish and seafood, meat, animal fat, dairy products, eggs and vegetable oils, Eljarrat et al. (2014) assessed the dietary daily exposure of the Spanish adult population to HBCDDs. The daily intake to the sum of α-, β- and γ-HBCDD was estimated at 2.58 ng/kg bw. The HBCDD exposure mainly came from fish and seafood (56%), dairy products (14%) and meat (12%).

In summary, the dietary exposure assessments performed in the past decade in European countries generally point to mean intake values for the sum of α-, β- and γ-HBCDD exposure between < 1 and 2.5 ng/kg bw per day, which is in general accordance with the exposure estimates performed by EFSA based on the occurrence data submitted by the European countries. The major categories contributing to total dietary exposure are food of animal origin, such as fish and fish derived products, meat, dairy products and eggs.

3.3.3. Non-dietary sources of 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 dust, and via dermal contact with consumer products that may contain HBCDDs. HBCDD levels in dust are summarised in **Appendix A**. In the former EFSA Opinion on HBCDDs, the CONTAM Panel concluded that 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 (EFSA CONTAM Panel, 2011a). The following paragraphs give some examples on non-dietary exposure assessments for HBCDDs published since the previous EFSA Opinion, and where available, compares the non-dietary with dietary exposure. The focus is on studies where the samples are collected after 2010.

Non-dietary oral exposure

Kalachova et al. (2012) determined several BFRs in dust collected in 25 Czech households and 27 car interiors (see levels **Appendix A**). The exposure was estimated assuming that the mean dust ingestion rates for adults and toddlers (6–24 months) were 20 and 50 mg per day, and high dust ingestion rates were 50 and 200 mg per day, respectively. For adults, and considering a mean household dust ingestion, the exposure (mean (median, P95)) was 2,600 (1,300, 8,600) pg per day, while for high dust ingestion it was 6,400 (3,300, 21,500) pg per day. The exposure from car dust was considerably lower: for a mean car dust ingestion the exposure was estimated as 50 (30, 130) pg per day, and for a high car dust ingestion, it was 110 (60, 320) pg per day. For toddlers, the exposure from both dust sources was higher. Considering a mean household dust ingestion, the exposure was estimated as 8,500 (4,400, 28,600) pg per day, and with a high household dust ingestion, it was 34,000 (17,700, 114,500) pg per day. As for adults, car dust exposure was lower: for a mean ingestion, the exposure was 110 (60, 320) pg per day, and for a high car dust ingestion, it was 460 (250, 1,300) pg per day.

Fromme et al. (2014) collected 20 residences' dust samples from vacuum cleaner bags and analysed them for several brominated flame retardants, including HBCDDs in order to assess human exposure through dust (see levels in **Appendix A**). Two scenarios were examined, an average intake on the basis of median concentrations in house dust, and a high intake on the basis of 95th percentiles. In addition, the exposure was determined for the group of adults and that of toddlers. Body weights of 70 kg and 12 kg for adults and toddlers, respectively, were assumed. The average daily intake of house dust was assumed to be 30 mg for adults and 60 mg for toddlers. The average and high intakes for adults are given as 0.15 pg/kg bw and 0.76 pg/kg bw, respectively. For toddlers, the respective daily intakes via dust amount to 1.7 pg/kg bw and 8.9 pg/kg bw.

Strid et al. (2014) investigated personnel exposure to BFRs both during flights and maintenance of aircrafts. High HBCDD concentrations in dust and air were detected in relation to the insulation material in the aircraft (see **Appendix A**), something that was not reflected in the serum samples of the involved personnel since the presence of HBCDDs was only indicated in a few samples. Allen et al. (2013) also investigated exposure to flame retardant chemicals from commercial aircraft and found levels in dust of up to 1,100,000 ng/g (see **Appendix A**).

Sahlström et al. (2015b) determined the concentrations of HBCDDs among other BFRs in diet and house dust for a mother–toddler cohort and estimated exposure via these two media. Dust samples (n = 27) were collected on surfaces at least 1 m above the floor in the living room, kitchen, bedroom and/or hall-way (see levels **Appendix A**). Based on dust ingestion rates of 30 mg per day for adults and 60 mg per day for toddlers, median (range) intakes for the sum of α-, β- and γ-HBCDD via dust of 3.4 (0.86–180) ng per day and 6.9 (1.7–360) ng per day for mothers and toddlers, respectively, were estimated. While for the mothers, the median dietary intake for the sum of the three HBCDD isomers was around three times higher compared to dust (11 vs. 3.4 ng per day), for toddlers, the median intake via dust exceeded the respective dietary intake (6.9 vs. 5.0 ng per day).

Coelho et al. (2016b) analysed 28 house dust samples (vacuum cleaner bags) from two Portuguese cities, for HBCDDs and other organic contaminants (see levels in **Appendix A**). Human exposure through dust ingestion was evaluated by calculating the estimated daily intakes based on the high dust ingestion rates of 100 mg per day for adults and 200 mg per day for children, and considering 70 and 12 kg as the average body weights of adults and children, respectively. While for adults, the estimated daily intake ranged from 22 to 2,900 pg/kg bw (mean: 540, median: 220 pg/kg bw), the corresponding intakes for children ranged from 260 to 33,000 pg/kg bw (mean: 6,300, median: 2,600 pg/kg bw).

Tay et al. (2017) estimated human exposure to HBCDDs and other flame retardants via inhalation and dust ingestion. For this assessment, 60 indoor stationary air samples, 13 personal air samples and 60 settled dust samples were collected from a Norwegian cohort during winter 2013 (see levels in **Appendix A**). The median (P95) daily exposure via dust ingestion was estimated as 68 (500) pg/kg bw. The estimated median (P95) daily inhalation from stationary air sampling and personal air sampling were 0.083 (4.2) pg/kg bw and 8.1 (14) pg/kg bw, respectively.

Besis et al. (2017) investigated the occurrence of brominated flame retardants in the dust from the interior of private cars in Thessaloniki/Greece, aged from 1 to 19 years with variable origin and characteristics (see levels in **Appendix A**). Median and 95th daily exposure to ∑HBCDDs through dust ingestion for adults were 0.00731 and 0.0253 ng/kg, respectively. For toddlers, the respective daily exposures were 0.0512 and 0.177 ng/kg bw. The median and 95th daily exposures through dermal contact for adults were 0.00310 and 0.0107 ng/kg bw, respectively. For toddlers, the respective daily exposures were 0.0402 and 0.139 ng/kg bw.

Kurt-Karakus et al. (2017) analysed several BFRs in indoor and outdoor air and indoor dust in urban, semi-urban and rural locations in Istanbul/Turkey, including HBCDDs (reported as γ-HBCDD) (see levels in **Appendix A**). The median low and high intake of HBCDDs via dust for adults and children was estimated to be 16–379 pg/kg bw per day and 952–3,460 pg/kg bw per day, respectively. The median intake via inhalation and dermal contact for adults and children were estimated as 25 and 6 pg/kg bw per day and as 5 and 12 pg/kg bw per day, respectively.

Larsson et al. (2018) analysed several brominated flame retardants in dust collected in 2015 from a total of 100 preschools in Sweden. In addition, also 100 hand wipe samples were collected and analysed for BFRs (see levels in **Appendix A**). For the exposure calculation of the 4-year-old children, a dust intake of 30 mg and a body weight of 17.6 kg was assumed. The daily intake of ∑HBCDD in preschool dust via dermal contact was calculated using various assumptions, such as exposed body area (hands, arms, legs) of children of 3,380 cm^2, amount of dust adhered to the skin, absorbtion factors and fraction of the time spent in the preschool. The estimated geometric mean (P95) daily

intake of ∑HBCDD in preschool dust via oral and dermal exposure in 4-year-old children were 200 (1,900) pg/kg bw and 7 (60) pg/kg bw, respectively.

Wemken et al. (2019) measured HBCDDs and other flame retardants in indoor air and dust collected from Irish homes, cars, offices and primary schools during 2016–2017. α-HBCDD was the dominant isomer in homes, offices and schools, with γ-HBCDD dominant in cars (see levels in **Appendix A**). Using median ∑HBCDD occurrence concentrations, the daily total exposure via air and dust for adults, toddlers and school children was estimated as 140, 2,500 and 1,400 pg/kg bw, respectively. Assuming ingestion/inhalation of the 95th percentile HBCDD concentrations, the respective daily exposure scenarios via air and dust was 7,800, 170,000 and 86,000 pg/kg bw.

The broad range of dust contamination with HBCDDs and its impact on non-dietary exposure is supported in the following investigations conducted in non-European countries.

Ali et al. (2012) analysed 50 indoor dust samples collected from different homes in selected rural and urban areas of New Zealand. HBCDD concentrations ranged between 20–4,100 ng/g (median: 190, mean 460 ng/g). For the exposure assessment, average adult and toddler dust ingestion figures of 20 and 50 mg per day, and high dust ingestion intakes for adults and toddlers of 50 and 200 mg per day, respectively,were assumed. Body weights used were 70 kg for adults and 12 kg for toddlers. Considering mean and high dust intake, the median (P95) daily exposure for adults was 50 (500) and 130 (1,260) pg/kg bw, respectively. For toddlers, the corresponding daily exposures were calculated as 780 (7,320) and 3,130 (29,300) pg/kg bw, respectively.

Tue et al. (2013) investigated the occurrence of several brominated flame retardants in indoor dust and air from two Vietnamese e-waste recycling sites (EWRSs) and an urban site in order to assess the relevance of these media for human exposure. The EWRSs were rural communes of approximately 250 households, with about 40–50% regularly involved in recycling of metals and plastics from e-waste. The levels of HBCDDs in settled house dust from the EWRSs of 5.4–400 ng/g were significantly higher than in dust from houses in urban and suburban areas which ranged from 0.99 to 61 ng/g, indicating the importance of informal recycling of e-waste.

Barghi et al. (2017) investigated BFR concentrations in 124 vacuum dust samples of six categories of indoor environments (homes, offices, kindergartens, cars, schools and public indoor environments) and 32 surface dust samples from five cities in South Korea, and based on these results, estimated exposures for adults and children. The median ΣHBCDD concentrations ranged from 106.30 ng/g in home dust to 496.13 ng/g in office dust. Due to the broad electrical equipment in offices, the measured concentrations were significantly higher than those determined in schools and homes ($p < 0.05$). The exposure via dust was estimated using the time activity in the different indoor places, and high and average dust intakes of 50 and 20 mg dust per day for 3-year-olds, and of 200 and 50 mg dust per day for 1–3 years olds. The estimated dust intake for adults and toddlers was 10.66 and 4.27 ng/day, and 35.75 and 8.94 ng/day, respectively. A comparison with dietary intake revealed that food is the major contributor for HBCDD intake in all subgroups except 1–3-year-olds in the high dust intake scenario.

Wang et al. (2018) analysed dust samples from 30 homes and 27 offices from Beijing (China) for a number of BFRs, including HBCDDs. While the concentrations for ∑HBCDD in house dust ranged from 73.6–995 ng/g (median: 156 ng/g), the respective concentrations in office dust were determined as 110–394 ng/g (median: 258 ng/g). The median exposure for ∑HBCDD via dust intake for adults was estimated as 48 pg/kg bw per day, which is substantially lower than the estimated daily dietary intake of 1,050 pg/kg bw per day based on a Chinese total diet study. For toddlers, the median intake via dust was reported as 649 pg/kg bw per day.

The CONTAM Panel made a rough scenario to estimate the potential ingestion from dust in toddlers and adults. The US-EPA assumed that while an adult ingests an average of 20 mg per day, a child of 1–2 years old ingests an average of 50 mg per day (US-EPA, 2017). Values found in dust from European countries ranged from very low to around 150,000 μg/kg in homes and up to 1,000,000 μg/kg in some vehicles (see **Section 1.3.3**). The majority of data reported for dust in homes had concentrations below 1,000 μg/kg, and so this was taken as a high but realistic value for ∑HBCDD to make an estimate of exposure from dust. Considering a body weight of 12 kg for a 1- to 3-year-old child and 70 kg for adults (EFSA Scientific Committee, 2012), the resulting daily exposures via dust ingestion would be around 4 ng/kg bw per day for toddlers and around 0.3 ng/kg bw per day for adults. Whilst there is a wide variation in exposure from dust reported in the literature (see above), this estimate is within the range of those reported. The CONTAM Panel noted that this is only a very crude estimate of the exposure via dust and has a large associated uncertainty, and that there is a wide range in estimates of exposure from dust reported in the scientific literature. It is nevertheless

evident that for all population groups, exposure from dust could make a substantial contribution to the overall HBCDDs exposure.

A further so far not intensively studied potential oral exposure can arise from unintentional ingestion of parts of plastic toys by small children. Fatunsin et al. (2020) analysed 23 plastic samples from 20 new and second-hand children's toys that had been previously shown to be bromine positive by XRF. HBCDDs were detected in 14 cases with concentrations between 0.25 and 840 mg/kg. Besides exposure from mouthing, exposure arising from accidental ingestion of plastic from toys can be significant for young children. Strakova et al. (2017) analysed several types of plastic toys for HBCDDs to examine if the recycling of e-waste plastics may lead to contamination of new products. The toys were initially screened for bromine using a handheld XRF analyser. Samples with substantial bromine levels were subsequently analysed for HBCDDs using ultra-high-performance liquid chromatography interfaced with tandem mass spectrometry with electrospray ionisation in negative mode (UHPLC-MS/MS-ESI). In total, 104 toy samples from 24 countries were analysed of which 45 samples (43%) contained HBCDDs at concentrations between 1 and 1,586 mg/kg. The highest concentration in products purchased in the EU amounted to 375 mg/kg in a toy gun. In a follow-up study, Strakova et al. (2018) analysed the black parts of 47 consumer goods (toys and hair accessories) bought in the Czech Republic for PBDEs and HBCDDs. Eleven samples contained HBCDDs at concentrations of 0.02–91 mg/kg. The authors concluded that the results indicate that HBCDDs found in e-waste are widely dispersed into children's toys made of recycled plastic due to unfavourable management of waste containing HBCDDs.

Dermal exposure

Pawar et al. (2017) investigated the dermal bioaccessibility of flame retardants from indoor dust and the influence of topically applied cosmetics. The authors performed an *in vitro* physiologically based extraction test of various BFRs from indoor dust to a synthetic sweat/sebum mixture (SSSM). The SSSM was prepared using over 25 different chemical components. In general, the bioaccessibility of HBCDDs increased with increasing sebum content of the SSSM. At 100% sweat, the bioaccessibility of γ-HBCDD (1.4 ± 0.1%) was less than that of β-HBCDD (1.6 ± 0.6%) and α-HBCDD (2.3 ± 0.2%). However, the reverse trend was observed at 100% sebum, where the bioaccessibility was highest for γ-HBCDD (67.2 ± 3.37%), followed by β-HBCDD (60.4 ± 10.1%) and α-HBCDD (50.5 ± 7.0%). This behaviour is consistent with the lower water solubility of the γ-stereoisomer compared with that of β-HBCDD and α-HBCDD. When tested, 6 mg of cosmetics (moisturising cream, sunscreen lotion, shower gel and body spray) were each examined separately. The presence of cosmetics decreased the bioaccessibility of HBCDDs from indoor dust, whereas shower gel and sunscreen lotion enhanced the bioaccessibility of HBCDD.

Miyake et al. (2010) performed a comparative study of human health risks posed by a flame retarded curtain with HBCDDs. The concentrations of α-HBCDD, β-HBCDD, γ-HBCDD and ΣHBCDDs in the curtain were 340,000, 150,000, 1,000,000 and 1,500,000 ng/g, respectively. The amounts of ΣHBCDDs, determined after drawing the curtain in triplicate, on the hand were 0.9 ± 0.6 ng. The lifetime average daily dose (LADD) by the dermal exposure via direct contact with the curtain was calculated as 0.0000025 mg/kg bw. The LADD by the ingestion exposure via hand-to-mouth contact assigning default values, such as contact frequency with the surface, hand-to-mouth events, dermal absorption efficiency and body weight, was calculated as 2.3×10^{-9} mg/kg bw. This value was a 100-fold lower than the LADD by the inhalation exposure of dust.

Tay et al. (2018) assessed dermal exposure to BFRs and compared direct measurements from hand wipes with an indirect estimation from settled dust concentrations. Sixty-one hand wipe samples were collected from a Norwegian adult cohort using gauze pads immersed in isopropanol. The range (median) of ΣHBCDDs in the hand wipe samples were 49–8,900 (180) ng per participant, corresponding to 27–11,000 (640) pg/cm^2 hand surface. A positive statistically ($p < 0.05$) correlation was found for γ-HBCDD between settled dust (ng/g) and hand wipe (ng/participant). Concentrations of ΣHBCDDs in hand wipes were positively correlated ($p < 0.05$) to the number of electronic consumer products at home (i.e. TV, DVD players, tablets, laptops, cell phones and PC screens). Positive correlations were also found between the number of people living in the house and the concentrations of α- and β-HBCDDs in hand wipes. The median (P95) daily dermal exposure to ΣHBCDDs was estimated as 840 (12,000) pg/kg bw per day.

An association of BFRs between children's hand wipes and house dust was also demonstrated by Stapleton et al. (2014). The ΣHBCDD levels in 43 hand wipes of children ranged from < 0.05–10.8 ng with a geometric mean of 0.97 ng. In the 30 house dust samples, the ΣHBCDD concentrations were

determined as 77.6–2,658 ng/g with a geometric mean of 338 ng/g. In general, higher levels of BFRs in house dust were associated with higher hand wipe levels.

Another potential source of dermal intake is the contact with used dishcloths. Gallistl et al. (2017) analysed 19 dishcloths after use for 14 days in kitchens for a number of polyhalogenated compounds. The median surface concentration for total-HBCDDs was determined as 550 (P95: 5,300) ng/m^2. Based on a skin absorption factor of 11–13%, a median intake of 5.4 (P95: 53) ng per day via skin was estimated.

In summary, the data on intake of HBCDDs via dust and dermal contact indicate that these routes of exposures may be substantial, especially for small children and toddlers and must be considered when total exposure to HBCDDs are assessed as they can exceed the dietary exposure.

3.4. Risk characterisation

The chronic human dietary intake that would lead to a body burden at the LOAEL of 0.9 mg/kg bw was estimated (see **Section 3.1.5.2**). The MOE was calculated by dividing this intake by the estimated dietary exposure (see **Section 3.3.1**). Although the LOAEL was identified from a study involving a single administration on PND10, it is not viewed as an acute effect because HBCDDs are persistent in the body, and an acute high level exposure is not likely to occur during the critical period of development of the human brain. PND10 marks the start of a critical period in the development of the rodent brain, which corresponds in humans to the period beginning in the third trimester of pregnancy and continues throughout the first 2 years of life.

As described in **Section 3.1.5.3**, an MOE higher than 24 would indicate a low health concern. The MOEs were calculated for all age groups across all food consumption surveys in the EFSA Consumption Database (see **Table 17**). The lowest MOE was 650, which was calculated from the UB high level dietary exposure in the toddler age group. The CONTAM Panel concluded that these MOE values do not raise a health concern.

Table 17: Margin of exposure (MOE) values across age groups[(a)] (rounded to two significant figures)

Age group	Mean					
	Min		Median		Max	
	LB	UB	LB	UB	LB	UB
Toddlers	17,000	3,700	7,100	2,400	3,000	1,600
Other children	21,000	6,400	8,400	3,100	3,700	1,900
Adolescents	34,000	12,000	14,000	6,600	7,000	3,700
Adults	29,000	14,000	15,000	8,100	8,400	5,300
Elderly	24,000	12,000	15,000	7,800	10,000	5,900
Very elderly	34,000	12,000	16,000	7,800	7,800	4,800
	P95					
Toddlers	4,100	1,500	2,600	1,100	1,000	650
Other children	6,400	2,300	3,000	1,400	1,200	800
Adolescents	10,000	4,600	5,300	2,700	2,000	1,500
Adults	8,100	4,900	5,100	3,200	2,000	1,700
Elderly	7,800	4,700	4,800	3,200	3,200	2,600
Very elderly	7,800	5,200	5,300	3,200	3,000	2,400

(a): It was not possible to assess the dietary exposure to HBCDDs for 'Infants' (not breastfed) using the data submitted to EFSA (see **Section 3.3.1**).

The CONTAM Panel noted that exposure to HBCDDs via dust and dermal contact can be an additional source of exposure to HBCDDs especially for children and can exceed dietary exposure (see **Section 3.3.3**).

For breastfed infants with average human milk consumption (800 mL per day) the LB and UB range of occurrence in pooled human milk samples would result in daily exposures (median, range) of 13.8 (2.3–73.4) ng/kg bw and 14.3 (3.2–73.7) ng/kg bw, respectively. The chronic human dietary intake that would lead to a body burden at the LOAEL of 0.9 mg/kg bw was estimated (see **Section 3.1.5.2**). The

MOE was calculated by dividing this intake by the exposure resulting from breastfeeding (see Section 3.3.1). This resulted in MOE values of 170 (1,022–32) and 164 (734–32), respectively. For infants with high milk consumption (1,200 mL per day), the LB and UB intakes are 20.7 (3.4–110.2) ng/kg bw and 21.5 (4.8–110.6) ng/kg bw, respectively. This resulted in MOE values of 114 (691–21) and 109 (490–21), respectively. The lowest MOE values for breastfed infants with high milk consumption and highest breast milk levels are below the value of 24 mentioned above. It was noted that since these were pooled samples, it was not possible to estimate specific values for individuals and MOE values will be lower for some of them. The Panel concluded that these MOE values may raise a health concern for some breastfed infants. These MOEs could only be increased by reducing the concentration of HBCDDs in breast milk by addressing exposure of the mother before and during lactation.

Comparison of body burdens in adults

In the previous Opinion on HBCDDs (EFSA CONTAM Panel, 2011a), the CONTAM Panel compared the body burden at the Reference Point calculated from animal studies with the estimated body burdens in humans as an additional approach to assess the health risk of exposure to HBCDDs in addition to the MOE calculated for exposure via the diet. For this comparison, the Panel considered at that time the available data on levels in human tissues, and in particular the levels in adipose tissue reported in the literature, as they were considered to best reflect long-term exposure to HBCDDs. The reported concentrations in adipose tissue in humans were converted to an overall body burden assuming an average fat content in adult women of 25% (van der Molen, 1998).

Following a similar approach, for the current assessment, the CONTAM Panel used the HBCDD results from the study of Malarvannan et al. (2013a) in visceral and abdominal fat of obese patients. In addition, serum data on HBCDDs from the study of Kalantzi et al. (2011) were converted to overall body burden. As the HBCDD levels in human breast milk are likely to be related to the body burden (on a lipid basis) of women of child-bearing age, the reported UB range for HBCDDs in human milk collected in European countries between 2014 and 2016 as part of the WHO/UNEP field studies were also converted to overall body burden. The CONTAM Panel is aware that the body fat content is dependent inter alia on gender, age and physical condition. Deurenberg et al. (2001) reported mean body fat concentrations of 31.2 ± 7.8% and 20.1 ± 7.6% for females (n = 234) and males (n = 182), respectively. In order to compare the results of the present assessment with the former HBCDDs Opinion (EFSA CONTAM Panel, 2011a), an average fat content of 25% was used. The results are depicted in **Table 18**. The MOE values were calculated by dividing the body burden at the LOAEL of 0.747 mg/kg bw by the estimated body burden based on the different human matrices.

Table 18: Overall body burden and margin of exposure (MOE) values for adults calculated on the basis of various human matrices (MOE values rounded to two significant figures)

Basis	Concentration range (medians) (ng/g lipid)	Body burden (μg/kg bw)	MOE
Adipose tissue	0.89–89 (4.1/3.7)[(a)]	0.223–22.3 (1.03/0.93)	3,400–34 (730, 810)
Serum	0.49–38.8 (1.32)[(b)]	0.123–9.7 (0.33)	6,100–77 (2,300)
Human milk	0.7–16.1 (3.12)[(c)]	0.175–4.03 (0.78)	4,300–190 (960)

(a): Data from Malarvannan et al. (2013a). Medians are for visceral and abdominal fat, respectively.
(b): Data from Kalantzi et al. (2011).
(c): UB range for HBCDDs in pooled human milk collected in European countries between 2014 and 2016 as part of the WHO/UNEP field studies (see **Table 8**).

The CONTAM Panel concluded that these results support the conclusion that current dietary exposure to HBCDDs in European countries does not raise a health concern.

3.5. Uncertainty analysis

The evaluation of the inherent uncertainties in the assessment of exposure to HBCDDs in food has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in the Dietary Exposure Assessment (EFSA, 2007). In addition, the report on 'Characterizing and Communicating Uncertainty in Exposure Assessment' has been considered (WHO/IPCS, 2008). The new guidance on uncertainties of the Scientific Committee (EFSA Scientific Committee, 2018) was not implemented in this Opinion as the draft was developed before the implementation of the guidance by the CONTAM Panel in its risk assessments.

3.5.1. Assessment objectives

The objectives of the assessment were clarified in **Section 1.2** on Interpretation of the Terms of Reference.

3.5.2. Exposure scenario/exposure model

Occurrence data

The exposure assessment was based on HBCDDs occurrence data reported by seven European countries. However, most of the data (78%) were reported by France and Germany. There is uncertainty around possible regional differences in HBCDDs contamination and the data set is likely not to be fully representative for the EU market.

Exclusion of the occurrence data reported as Total HBCDDs (analysed by GC-MS) due to the high LOQ values and high proportion of left-censored data resulted in excluding three food categories: 'Vegetable oils', 'Ready-to-eat meal for infants and young children' and 'Dietary supplements', which may result in underestimation of exposure (see **Annex B**, Table B.5). In case of occurrence data expressed on a fat weight basis, the contamination level was combined with the fat content as reported for the related sample. Where the fat content was missing, the median value of the samples reported for the same category was used. This could lead to both over- and underestimation of the exposure. In addition, exclusion of the food categories 'Infant formulae', 'Follow-on formulae' and 'Cereal-based food for infants and young children' due to the data reported for the individual stereoisomers being 100% left-censored, may lead to an underestimation of dietary exposure of 'Toddlers' to HBCDDs. This did not allow to estimate the exposure of 'Infants' from food.

A high proportion of left-censored data (71%) was reported across all food categories including the main contributors to the dietary exposure and consequently the difference between LB and UB estimates is two- to threefold. The use of the LB in this Opinion tends to underestimate, while the UB tends to overestimate the dietary exposure. The limited number of available analytical results for some food categories adds uncertainty to the representativeness of the mean concentration values used to estimate the exposure. For some highly consumed food groups ('animal fat', 'liquid milk'), the sensitivity of the methods seems too low, resulting in a large uncertainty in the exposure from these food groups, thereby increasing the difference between LB and UB exposure.

Exposure to HBCDDs from food supplements containing special fatty acids (e.g. fish oil) could not be considered due to lack of occurrence data. Moreover, the contribution from several food categories, especially of plant origin, could not be considered due to lack of data. This will result in an underestimation of exposure.

Consumption data

Uncertainties and limitations related to the use of the EFSA Comprehensive Food Consumption Database have already been described by EFSA (EFSA, 2011a) and are not further detailed in this Opinion. Generally, these limitations and uncertainties relate to the use of different dietary survey methodologies, standard portion sizes, representativeness of samples included in surveys, or to the inclusion of consumption surveys covering only few days to estimate high percentiles of chronic exposure.

Dietary Exposure Assessment

Important sources of uncertainty are related to the different assumptions done, mainly for the linkage between the occurrence and consumption data. In particular:

- Sampled foods were assumed to represent consumed foods, which could lead to both over- and underestimation of the exposure.
- A number of foodstuffs were grouped mainly at the 2nd level of the FoodEx system, assuming homogeneity of the contamination levels. This was the case for food categories represented with less than six samples or 100% left-censored at the third level, but enough data were available at the upper level. This could lead to both over- and underestimation of the exposure.
- For the samples reported as 'Salmon and trout (Salmo spp.)' without additional information on the species, and therefore the reclassification not possible, homogeneity of the contamination was assumed. This could lead to both over- and underestimation of the exposure.

Chronic dietary exposure is based on the sum of the three major HBCDD stereoisomers (α-, β- and γ-HBCDD) for all food categories. Due to the exclusion of δ- and ε-HBCDD, exposure might be slightly underestimated. The CONTAM Panel noted that these stereoisomers δ- and ε-HBCDD are only present at low concentrations in the technical product, and in food.

The exposure of breastfed infants was estimated based on pooled samples from several European countries, showing a wide range of concentrations. Since these were pooled samples, it was not possible to estimate the distribution within the population. Some breastfed infants showed an exposure that resulted in an MOE smaller than 24, based on high milk intake and HBCDD levels in pooled milk. This may imply a health concern, also considering that HBCDD levels in milk of individual mothers are likely to be higher than that in the pooled sample. This higher exposure is caused by the body burden of the mothers due to chronic exposure. Smaller MOEs in these infants may be in contrast to the large MOEs for adults, even at the P95 (1,700–8,100). The transfer to infants should be taken into account when deriving the safe intake level in humans but there is currently too little information to base the risk assessment on the body burdens of mothers and transfer to the infant.

Effect of cooking/processing was not taken into account. The human exposure estimations in this Opinion are based on consumption data and occurrence levels in raw food commodities. It is known that typical household cooking practices neither lead to degradation nor to generation of appreciable amounts of HBCDDs. However, changes in the fat content of food commodities during cooking/processing practices may lead to changes of the lipophilic contaminants in the processed food compared to the raw food commodity. Due to lack of representative data on these possible changes the effect of cooking and processing could not be taken into account. This may have added to the uncertainty in the exposure estimation to some extent.

3.5.3. Hazard identification and characterisation

No data were identified regarding absorption in human, and sparse data were found on distribution and metabolism.

Only one study reported on the terminal elimination half-life of HBCDDs in humans. Geyer et al. (2004, extended abstract) estimated a half-life of 64 days (range 23–219 days) by using several estimations (daily intake, fat mass). However, the method is not fully described. This leads to uncertainties regarding the human half-life value as follows:

- The authors indicated that '*the whole body (total body burden) half-lives in humans (t½H in days) of BFRs were estimated from the daily intake (DI in ng per day -1) and the total body burden under steady state conditions in non-occupationally exposed adult humans*'. It is not clear for the CONTAM Panel if the estimate of daily intake of HBCDDs corresponded to the same population in which HBCDD concentrations were measured.
- The demographic characteristics (number of subjects, weight, age, gender etc.) are not provided. According to the equation used by Geyer et al. (2004, extended abstract), the human half-life was estimated by considering a fat mass value (m_f) of 13.5 kg for adult man and 18.7 kg for an adult woman:

$$t_{1/2H} = \frac{\ln 2 \times m_{ss}}{DI \times f} = \frac{0.693 \times c_{ss} \times m_f}{DI \times f}$$

where f is the fraction of dose absorbed from food, m_{ss} is the total amount (in ng) of the chemical in the whole human body at steady state, c_{ss} is the concentration (ng/kg lipid) in adult humans and m_f is the fat mass (13.5 kg for an average adult man and 18.7 kg for an average adult woman).

The authors assumed that the HBCDD concentrations were converted into total body burdens assuming a balance between all lipid compartments in the body. Nevertheless, the fat content of the human body varies with age, sex, weight and height, as previously described by Deurenberg et al. (1991). The CONTAM Panel noted that information about the demographic characteristics data on the subject used for the estimation was not available.

- In their equation, Geyer et al. (2004, extended abstract) used as internal dose metric the concentration (ng/kg lipid) in adult humans measured in breast milk. Further information on how the calculation of the half-life was performed is not available.

As noted in the previous Opinion (EFSA CONTAM Panel, 2011a), most of the toxicological studies with HBCDDs were performed with HBCDDs preparations for which the purity and stereoisomer composition was not always indicated, rather than with purified individual stereoisomers. As the toxicity of the individual stereoisomers is not known, and the toxicological Reference Point is based on information from exposure to HBCDDs as mixtures of isomers with unclear relevance to the distribution of isomers in food, this adds a considerable uncertainty to the risk assessment.

The studies on neurobehavioural effects of HBCDDs all have limitations, which introduce uncertainty into the Reference Point. The studies of Eriksson et al. (2006) and Zhang et al. (2017a) did not assess anxiety and swim speed, although these parameters might also explain the increase in latency to reach the platform in the Morris Water Maze. Thus, there is uncertainty as to whether HBCDDs have effects on spatial learning and memory. Changes in the anxiety level and horizontal locomotion could also explain observed effects on spontaneous behaviour as recorded in Eriksson et al. (2006).

In the Eriksson et al. (2006) study, a single dose of HBCDDs was administered to mice on PND10, which marks the start of a critical period in the development of the rodent brain. It is unclear whether PND10 is the most critical day and if exposure at another time point would produce a response at a lower dose.

There is also uncertainty as to whether an effect would have been observed at a lower dose following repeated dosing. However, the approach used in the current Opinion is conservative, as the estimated body burden suggest that repeated dosing would provide a higher body burden than that calculated with single exposure at the same initial dose, and therefore a higher reference point.

Although occurring at a dose (49.5 μg/kg bw per day) below that used to derive a Reference Point, the CONTAM Panel chose not to further consider effects on sex hormones due to a lack of clear dose response and information on related apical effects at the LOEL (Maranghi et al., 2013; Rasinger et al., 2014, 2018). Likewise, effects on metabolism, and in particular lipid metabolism, were observed at a dose of 7.1 μg/kg bw per day (Xie et al., 2019), but because of uncertainty regarding relevance to human health, this endpoint was also not selected for risk characterisation. There is remaining uncertainty regarding the relevance of these findings and therefore of the selected endpoints for establishment of a Reference Point.

Exposure of adult rats caused an increase in hepatic glucuronidation of T4 hormone (van der Ven et al., 2006). Whilst increased T4-UGT was only significant at a relatively high dose of 100 mg/kg per day, the authors reported a $BMDL_{10}$ of 4.1 mg/kg per day (van der Ven et al., 2006). There is an uncertainty as to whether this effect on T4 conjugation might be different in early infancy and, thus, whether effects on neurodevelopment might be secondary to changes in thyroid hormone homeostasis. Furthermore, there is uncertainty about the relevance of glucuronidation for thyroid hormone homeostasis in humans.

The CONTAM Panel conducted BMD modelling on the neurobehavioural data of Eriksson et al. (2006) according to the latest EFSA guidance (EFSA Scientific Committee, 2017), and found that the BMD confidence intervals were relatively wide and that the BMDLs for horizontal locomotion and total activity were far below the lowest dose administered. Therefore, the NOAEL/LOAEL approach was applied, since the BMD modelling generally indicated that determination of the Reference Point based on the key data is uncertain.

Regarding the available epidemiological evidence, the assessed evidence base is characterised by relatively small studies, heterogeneous populations and lack of replicated endpoints (no studies identified on the same endpoint). Therefore, there is uncertainty as to whether the observed epidemiological associations are causal.

3.5.4. Summary of uncertainties

In **Table 19**, a summary of the uncertainty evaluation is presented, highlighting the main sources of uncertainty and indicating an estimate of whether the respective source of uncertainty might have led to an over- or underestimation of the exposure or the resulting risk.

Table 19: Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of HBCDDs in food

Sources of uncertainty	Direction
Hazard identification and characterisation	
Limited information on the stereoisomer composition and purity of the HBCDDs used in toxicological studies	+/–
Isomeric profiles of HBCDDs used in toxicological studies are not the same as the profiles found in food	+/–
Limited data on the toxicokinetics of HBCDDs in humans, including uncertainty in the data on the half-life of HBCDDs in humans	+/–
Lack of information on the toxicity of the individual stereoisomers	+/–
Whether exposure on a day other than PND10 in mice would produce a response at a lower body burden	–
Whether repeated exposure of HBCDDs will produce neurobehavioural effects in rodents at a lower body burden	–
Lack of large-scale prospective epidemiological data to support the toxicological effects	+/–
Determination of the Reference Point based on the key data is uncertain	+/–
Occurrence and exposure assessment	
Uncertainty of analytical results due to different LODs/LOQs	+/–
Some food categories could not be considered due to lack of occurrence data	–
Extrapolation of occurrence data from a few countries to whole Europe	+/–
Impact of upper-bounds for non-detects on dietary exposure estimate	+
Distribution of HBCDDs levels in individual human milk samples is not known because only pooled samples analysed across Europe	+/–
Lack of information on the impact of food processing	+/–
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–

(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 HBCDDs in food is substantial. Without a quantitative uncertainty analysis, the Panel was unable to determine the direction of the overall uncertainty.

4. Conclusions

HBCDDs, composed of mixtures of stereoisomers of 1,2,5,6,9,10-hexabromocyclododecane, were widely used as additive flame retardants in a number of applications. Due to the widespread use, HBCDDs have found global distribution. HBCDDs were permitted to be used in the EU until August 2015, after which only authorised applications were allowed because of health concerns.

The present assessment is an update of the former EFSA CONTAM Panel Opinion on HBCDDs published by EFSA in 2011. It takes into account the occurrence data in food and biological samples submitted after the publication of the former Opinion, as well as the newly available scientific information on hazard identification and characterisation.

The analytical determination of HBCDDs is performed either by GC/MS or LC/MS-based methods. Both techniques enable the use of isotope-labelled internal standards, which allow for the correction for losses during extraction and clean-up. While GC/MS-based methods determine Total HBCDDs as they cannot separate the stereoisomers, LC/MS-based methods allow the specific analysis of the individual HBCDD stereoisomers.

4.1. Hazard identification and characterisation

4.1.1. Toxicokinetics

- In rodents, absorption of HBCDD stereoisomers from the gastrointestinal tract is rapid and almost complete (> 80%).

- HBCDDs and/or their metabolites are distributed to a number of tissues, including adipose tissue, muscle, liver, skin and brain. Following a single oral administration in rats, α-HBCDD and β-HBCDD accumulate in adipose tissue, while γ-HBCDD does in liver and adipose tissue. In repeated dose experiments in mice, α-HBCDD and β-HBCDD accumulate mainly in adipose tissue, while γ-HBCDD accumulates in the liver.
- Metabolic debromination and hydroxylation of HBCDDs have been reported.
- Conversion of γ-HBCDD to α- and β-HBCDD has been reported, but no stereoisomerisation of α-HBCDD.
- Elimination of HBCDD stereoisomers in rodents is predominantly via faeces. The elimination half-lives of HBCDD stereoisomers in female mice, based on concentrations in adipose tissue, vary from 3–4 days for γ-HBCDD, 2.5 days for β-HBCDD and 17 days for α-HBCDD.
- In humans, in the only available study, the half-life was estimated to be 64 days (range 23–219 days) for the sum of α-, β- and γ-HBCDD stereoisomers. This difference in kinetics affects the extrapolation of animal data to humans.
- Transfer of HBCDDs was studied in different animal species (laying hens, broilers, ducks, pigs and fish). HBCDDs are distributed to a number of tissues and accumulate in tissues with high-fat content and eggs.

4.1.2. Toxicity in experimental animals

- The acute toxicity of HBCDDs is low.
- Toxicological studies have been carried out using different experimental designs with single or repeated administration during gestation, postnatally or in adulthood using, in most cases, HBCDDs with no information on the stereoisomer composition specified. Main targets for toxicity are neurodevelopment, the liver, thyroid hormone homeostasis and the reproductive and immune systems.
- HBCDDs induce increased liver weight and hepatocellular hypertrophy in rats and mice. Increased thyroid weight, follicular hypertrophy, hyperplasia and/or colloid depletion and changes in thyroid hormone levels were also reported in these species.
- HBCDDs decreased the fertility index and increased pup mortality during lactation in a 2-generation toxicity study in rats. In addition, HBCDDs decreased testes weight and delayed vaginal opening in female pups in a 1-generation study in rats. Other endocrine-related effects are changes in levels of sex steroid hormones (testosterone and oestradiol) in mice, decreased number of primordial ovarian follicles or reduction in the number of growing follicles in rats.
- HBCDDs affected the immune system. Changes observed included decreased thymus weight and increased lesions in the thymus, reduced splenocyte proliferation and increased spleen lesions, decreased T-cell and increased B-cell populations, increased NK cells and changes in immunoglobulin responses.
- Exposure of juvenile rats and mice to HBCDDs caused neurodevelopmental effects on behaviour, leading to changes in spontaneous behaviour, spatial learning and memory as detected in adulthood.
- HBCDDs are not genotoxic *in vitro* or *in vivo*. The slight induction of DNA strand breaks observed in some *in vitro* tests is most likely due to oxidative stress.
- Based on the available information to the CONTAM Panel, i.e. the lack of carcinogenicity in the mouse study, the lack of direct genotoxicity and the information available on mode of action, there is no indication that HBCDDs are carcinogenic.

4.1.3. Observations in humans

- A growing number of epidemiological publications were identified assessing the association between exposure to HBCDDs and birth weight/length, neurodevelopment and thyroid dysfunction in children, as well as subfertility, type 2 diabetes, thyroid hormone levels, severe endometriosis (including ovarian endometrioma) and breast cancer metastasis in adults.
- None of the effects studied in the longitudinal studies and assessing internal exposure either reached statistical significance or were replicated in a longer follow-up point in the same study. Considerable limitations exist pertaining to small sample sizes, varying methodological quality, effect inconsistency and considerable heterogeneity in the assessed populations, exposures and endpoints.

- Adverse effects of HBCDDs related to neurodevelopment have been assessed in two epidemiological studies; the low volume of prospective data, the differing endpoint measures and the lack of replication render these data insufficient for use in risk characterisation.

4.1.4. Mode of action

- Effects of HBCDDs on the liver of rodents appear to involve CAR and PXR. Mitochondrial energy production is reduced with downstream effects on reactive oxygen species (ROS) production, Na^+/K^+-ATPase and Ca^{2+}-ATPase activities and increased cytosolic [Ca^{2+}] and [Zn^{2+}].
- HBCDDs stimulate proliferation and migration of liver cell lines at picomolar to nanomolar concentrations and there is evidence that these effects are related to activation of the PI3K/Akt/mTOR and oestrogen receptor signalling pathways. These effects might contribute along with activation of CAR, PXR and PPARs to the observed increase in liver weight in rodent studies with HBCDDs.
- Oral HBCDD exposure of mice fed a high-fat diet aggravates metabolic dysfunction through modifications of lipid and glucose homeostasis.
- HBCDD-induced lipid accumulation in liver and adipose tissue appears to be associated with suppression of the Wnt/β-catenin pathway resulting in increased expression of *Pparg* and its adipogenic target genes.
- Mechanistic studies on the effects of HBCDDs on the nervous system support specific effects on dopaminergic and glutamatergic neurons. This appears to include inhibition of dopamine and glutamate reuptake following synaptic release, and deregulation of Ca^{2+} and Zn^{2+}. The brain could also be affected by HBCDDs through diminished responsiveness to thyroid hormones.
- HBCDDs may affect the synthesis of sex steroids hormones by interfering with cAMP-dependent cholesterol uptake and by causing dysregulation of enzymes involved in sex steroid metabolism. HBCDDs may also alter the response of tissues to sex steroids.
- The mechanism(s) involved in HBCDD-induced ROS production may be related to impairment of mitochondrial energy production.

4.1.5. Critical effects and dose–response analysis

- The evidence from the available human data was not sufficient to base the risk assessment on. Thus, the data from studies on experimental animals were used to identify a Reference Point for the human health risk characterisation.
- The CONTAM Panel concluded that the neurodevelopmental effects on behaviour can be considered the critical effect for the risk characterisation.
- The BMD modelling on the neurobehavioural data showed BMD confidence intervals that were relatively wide and that the BMDLs for horizontal locomotion and total activity were far below the lowest dose administered. Therefore, the NOAEL/LOAEL approach was applied.
- Based on effects on spontaneous behaviour (horizontal locomotion, rearing and total activity), the CONTAM Panel identified an LOAEL of 0.9 mg/kg bw (single dose) as the Reference Point for the risk assessment of HBCDDs.
- Because the elimination kinetics for HBCDDs between mice and humans differ, the CONTAM Panel first calculated a body burden of 0.75 mg/kg bw at the LOAEL, considering an oral absorption of 83% in mice.
- The chronic intake that would lead to the same body burden in humans was calculated assuming an absorption in humans of 100% and the longest half-life identified in humans for HBCDDs of 219 days. This resulted in an estimated chronic human dietary intake of 2.35 μg/kg bw per day.
- Due to limitations in the database on HBCDDs, the derivation of a health-based guidance value (HBGV) was not considered appropriate. Instead, the margin of exposure (MOE) approach was applied to assess possible health concerns.
- The CONTAM Panel considered that an MOE higher than 24 would indicate a low health concern.

4.2. Occurrence and exposure for the European population

4.2.1. Occurrence in food

- A total of 6,857 analytical results for HBCDDs in food fulfilled the quality criteria applied and from these 6,352 were selected and used in the assessment after grouping the categories and matching with the food consumption data.
- Contrary to the data used in the previous Opinion on HBCDDs published in 2011, where most of the data were analysed with GC/MS and reported Total HBCDDs, the current data have mostly been analysed by LC/MS and reported on the specific stereoisomers α-, β- and γ-HBCDD.
- The high proportion of left-censored data, partially due to relatively high LOQs, resulted in a large difference between the lower bound (LB) and upper bound (UB) occurrence values for many food groups.
- The highest mean concentrations of HBCDDs (sum of α-, β- and γ-HBCDD) were recorded for the food category 'Fish meat', with the highest mean concentrations in eel, being 2.33 and 2.35 μg/kg ww at LB and UB, respectively.

4.2.2. Exposure assessment

- The mean dietary exposure estimates for HBCDDs ranged from 0.07 (minimum LB)/0.17 (minimum UB) to 0.79 (maximum LB)/1.52 (maximum UB) ng/kg bw per day across dietary surveys and age groups. At the 95th percentile, dietary exposure estimates ranged from 0.23 (minimum LB)/0.45 (minimum UB) to 2.30 (maximum LB)/3.61 (maximum UB) ng/kg bw per day.
- The high proportion of left-censored data, partially due to relatively high LOQs, resulted in a two- to threefold difference between the LB and UB exposure estimates.
- The most important contributors to the chronic dietary LB exposure to HBCDDs were 'Fish meat', 'Eggs, fresh', 'Livestock meat' and 'Poultry'.
- The exposure scenario based on average human milk consumption and the reported UB range for HBCDDs (sum of α-, β- and γ-HBCDD) in pooled human milk samples collected in European countries between 2014 and 2016 as part of the WHO/UNEP field studies, would result in a median daily exposure of 14.3 ng/kg bw (range: 3.2–73.7 ng/kg bw). For infants with high human milk consumption, the median daily exposure would result in 21.5 ng/kg bw (range 4.8–110.6 ng/kg bw).
- Non-dietary exposure through intake of dust and dermal contact 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.

4.3. Risk characterisation

- MOE values were calculated by comparison of the calculated chronic human dietary intake of 2.35 μg/kg bw per day, leading to the body burden at the LOAEL, with the estimated dietary exposure for the different population groups. The MOE values obtained ranged from 34,000 to 650. These MOEs are larger than 24 and the CONTAM Panel concluded that they do not raise a health concern.
- The CONTAM Panel also compared the body burden of 0.75 mg/kg bw at the LOAEL with the body burdens in adults based on levels in adipose tissue, blood and milk reported in the literature. The results support the conclusion that current dietary exposure to HBCDDs in European countries does not raise a health concern.
- For breastfed infants, the lowest MOE values for high milk consumption are below the value of 24. The CONTAM Panel concluded that these MOEs may raise a health concern for some breastfed infants.

5. Recommendations

In order to improve the risk assessment and reduce the uncertainties, the CONTAM Panel made the following recommendations:

- Criteria for the analysis of HBCDD stereoisomers should be set.
- Surveillance of HBCDD stereoisomers in food, in particular in food groups for infants and small children, should continue in order to refine the exposure estimates.
- More data on occurrence of HBCDDs in human milk are needed to enable a more robust exposure assessment for breastfed infants.
- Improved information on toxicokinetics, e.g. half-life values, of HBCDD stereoisomers in humans is needed. More information is needed on the body burden in mothers and relation to transfer of HBCDDs to milk. This information should be used to develop a toxicokinetic model for HBCDDs, including excretion into breast milk and placental transfer.
- More information is needed on the transfer into ruminant meat and milk.
- Further toxicological studies should be conducted with individual HBCDD stereoisomers most relevant to human exposure.
- Studies on neurodevelopment (after repeated exposure), including investigations of the mechanisms and mode of action involved, are recommended.
- Studies on reproductive effects are recommended.
- Studies on possible diabetogenic and obesogenic effects are recommended.
- Longitudinal epidemiological studies of sufficient power and appropriate exposure and co-exposure assessment are needed.

Documentation provided to EFSA

1) Malisch R and Schächtele A, 2019. Data provided to EFSA by Rainer Malisch and Alexander Schächtele on the HBCDDs-related results of the WHO/UNEP coordinated exposure studies 2000–2015 performed in European countries, and used in **Section 3.1.1.4**.

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Abbreviations

AMPK	Adenosine monophosphate-activated kinase
APCI	Atmospheric pressure chemical ionisation
APPI	Atmospheric pressure photoionisation
BDNF	Brain-derived neurotropic factor
BFRs	Brominated Flame Retardants
BMD	Benchmark dose
BMDL	Benchmark dose lower confidence limit
$BMDL_{10}$	Benchmark dose lower confidence limit for a benchmark response of 10%
BMI	Body mass index
BMR	Benchmark response
CAR	Constitutive androstane receptor
CDH1	Suppressed E-cadherin
CYP	Cytochrome P450
DNA-PKcs	DNA-dependent protein kinase
DTT	Dithiothreitol
dw	dry weight
E2	Estradiol
EF	Enantiomeric fraction
EPS	Expanded polystyrene foams
EQS	Environmental quality standard
ESI	Electrospray ionisation
EURL	European Reference Laboratory
FE2	Free estradiol
FGF	Fibroblast growth factor
FSH	Follicle stimulating hormone
GC	Gas chromatography
GIC	Groningen Infant COMPARE birth cohort
HBCDDs	Hexabromocyclododecanes
hCG	human chorionic gonadotropin
HSD17β	17β-hydroxysteroid dehydrogenase
IC50	half maximal inhibitory concentration
i.p.	intraperitoneal
InhB	inhibin B
IQR	median interquartile range
IRIS	Integrated Risk Information System
LADD	Lifetime average daily dose
LB	lower bound
LC	Liquid chromatography
LH	Luteinising hormone
LOAEL	Lowest observed adverse effect level
LOD	Limit of detection
LOEL	Lowest-observed-effect level
LRMS	low resolution MS
MAPK	Mitogen-activated protein kinases
MDL	Method detection limit

MOE	Margin of exposure
MMP9	Matrix metalloprotein 9
MS	Mass spectrometry
MSC	Mesenchymal stem cells
mTOR	Mammalian target of rapamycin
NGF	Nerve growth factor
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NIPH	Norwegian Institute of Public Health
NITE	Japanese National Institute of Technology and Evaluation
NMDA	N-methyl-d-aspartate
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
NRLs	National Reference Laboratories
PBDEs	Polybrominated diphenyl ethers
PBPK	Physiologically based pharmacokinetic
PBT	Persistent, bioaccumulative and toxic
PCBs	Polychlorinated biphenyls
PCNA	proliferating cell nuclear antigen
PI3K	Phosphatidylinositide 3-kinase
PLE	Pressurised liquid extraction
PND	Postnatal day
POPRC	Persistent Organic Pollutants Review Committee
POPs	Persistent Organic Pollutants
PPAR	Peroxisome proliferator-activated receptors
PXR	Pregnane-X-receptor
ROS	Reactive oxygen species
SERCA	Sarcoplasmic-endoplasmic reticulum Ca^{2+}-ATPase
SHBG	Sex hormone-binding globulin
SPE	Solid phase extraction
SSD	Standard Sample Description
SSSM	Synthetic sweat/sebum mixture
SVHC	Substances of very high concern
T2D	Type 2 diabetes
TBBPA	Tetrabromobisphenol A
TDS	Total Diet Study
TOF	Time-of-flight
UB	Upper bound
UK	United Kingdom
UNEP	United Nations Environment Programme
UPLC	Ultra performance LC
UV	Ultraviolet
WHO	World health Organisation
ww	wet weight
XPS	Extruded polystyrene foams

Appendix A – Levels in dust

Table A.1: Occurrence of HBCDDs in dust in Europe

Country	Sampling year	Sampling place	ΣHBCDD	α-HBCDD	β-HBCDD	γ-HBCDD	Reference
			Median (range) (ng/g)				
Sweden	2006	Houses	100 (15–990)	–	–	–	de Wit et al. (2012)
		Apartments	45 (< 3–2,400)				
		Offices	300 (190–1,600)				
		Day-care centres	340 (190–1,600)				
		Cars	54 (6.8–170)				
Sweden	2008	Vacuum cleaner bags	89 (6.4–95,000)	–	–	–	Björklund et al. (2012)
		Researcher-collected dust	8.9 (5.9–62)				
Sweden	2015	Preschool	100 (7.6–16,000)	52 (4.9–3,200)	15 (1.2–1,200)	26 (1.3–12,000)	Larsson et al. (2018)
Sweden	2012	Offices, apartments, stores and schools	150 (17–2,900)	–	–	–	Newton et al. (2015)
Sweden	2010–2011	House dust	110 (20–6,000)	56 (14–1,400)	18 (3.4–730)	37 (2.5–4,000)	Sahlström et al. (2015b)
Sweden	2006	Houses	100 (15–990)	–	–	–	Thuresson et al. (2012)
		Apartments	45 (< 3–2,400)				
		Offices	300 (190–5,700)				
		Day-care centres	340 (190–1,600)				
		Cars	54 (6.8–170)				
Norway	2013–2014	Homes	190 (0.43–150,000)	94 (< 0.14–74,000)	23 (< 0.017–60,000)	43 (< 0.28–13,000)	Tay et al. (2017)
Germany	2010	House dust	345 (53–4,041)	180 (32–1,063)	35 (8–496)	114 (14–3,563)	Fromme et al. (2014)

Country	Sampling year	Sampling place	ΣHBCDD	α-HBCDD	β-HBCDD	γ-HBCDD	Reference
			Median (range) (ng/g)				
France	2008	Houses	1,125 (636–1,865)	559	144	422	Abdallah et al. (2016)
		Offices	4,493 (1,096–10,188)	2,722	442	1,329	
		Cars	4,539 (1,458–7,900)	2,221	629	1,689	
Belgium	2008	House dust	130 (5–42,692)	–	–	–	D'Hollander et al. (2010)
		Office dust	367 (256–1,153)				
UK		Kitchen	–	110 (5.2–3,800)	29 (2.3–1,100)	35 (1.7–13,000)	Kuang et al. (2016)
		Living room/ bedroom	–	280 (75–4,900)	67 (6.4–1,600)	110 (14–21,000)	
		Matched kitchen-living room/ bedroom dust	–	0.37 (0.05–2.88)	0.41 (0.08–1.86)	0.37 (0.003–34.85)	
UK		Homes	610 (50–110,000)	320 (21–28,000)	85 (6.1–12,000)	93 (23–71,000)	Tao et al. (2016a)
		Offices	1,700 (150–6,400)	980 (100–2,800)	330 (22–590)	350 (31–3,700)	
Ireland	2016–2017	Office dust	380	–	–	–	Wemken et al. (2019)
		School dust	800				
		Car dust	490				
Portugal	2010–2011	House dust	150 (16–2,000)	91 (11–1,800)	16 (1.9–230)	35 (2.8–900)	Coelho et al. (2016b)
Czech Republic	2008	Car dust	(< 0.3–241)	(< 0.3–45)	(< 0.3–44)	(< 0.3–152)	Kalachova et al. (2012)
		Household dust	(< 0.3–950)	(< 0.3–275)	(< 0.3–57)	(< 0.3–740)	
Czech Republic	2013	House dust	122 (34.1–714)	–	–	–	Lankova et al. (2015)
Romania	2010	Indoor dust	325 (4–2,190)	200 (4–1,325)	30 (<LOQ–165)	55 (< LOQ–1,910)	Dirtu et al. (2012)
Greece	2016	Car dust	–	90.3 (< 5–1,288)	15.8 (< 5–294)	46.4 (< 5–260)	Besis et al. (2017)

Country	Sampling year	Sampling place	ΣHBCDD	α-HBCDD	β-HBCDD	γ-HBCDD	Reference
			Median (range) (ng/g)				
Istanbul/ Turkey	2012	*Urban location* Office dust Home dust	(140–94,000) (50–8,800)	–	–	–	Kurt-Karakus et al. (2017)
		Semi-urban location Office dust Home dust	(< LOD–20,000) (110–1,200)				
		Rural location Office dust Home dust	Not analysed (230–29,000)				
Airplanes	2010	Floor	7,600 (180–1,100,000)	2,300 (4.7–290,000)	310 (1.2–75,000)	4,500 (130–700,000)	Allen et al. (2013)
		Vent	10,000 (370–97,000)	1600 (17–32,000)	230 (0.8–11,000)	7,600 (99–59,000)	
Airplanes	2012	During maintenance work in aircraft	(301.6–231,010)	–	–	–	Strid et al. (2014)
		During flight (ventilation outlets in aircraft toilets)	(243.85–173,260)				
Several countries	2002–2017	Dust (children's indoor exposure)	World-wide dust levels: ranging from 6 (Egypt) to 340 ng/g (Sweden)	–	–	–	Malliari and Kalantzi (2017)

Appendix B – Literature search to identify studies on HBCDDs

The details of the literature searches performed to identify studies on HBCDDs are shown in **Table** B.1 The search strings were run in the databases indicated, and the CAS numbers in SciFinder.

The literature searches were performed in February 2019. The outcome of the searches in the different databases was saved in separate EndNote files and an automatic duplicate detection run. The references were then transferred to DistillerSR (a web-based systematic review software) where another automatic duplicate detection was made (**Table** B.2). The selection for relevance based on title and abstract and full text was done in duplicate by EFSA staff.

An update of the literature available in the public domain was done in April 2020, and since that date, the literature was monitored to identify studies relevant for the risk assessment until the time of endorsement.

Table B.1: Details of the literature search for HBCDDs

Databases interrogated:	WoS – Web of Science PubMED SciFINDER (including CAS numbers)
Year:	2010-date of the search
Languages:	English
Type of documents:	Peer reviewed articles, reviews, books
Search string:	TS=(hexabromocyclododecane OR hexabromocyclododecane* OR HBCDD OR HBCDDs OR *hexabromocyclododecane OR alpha-hexabromocyclododecane OR beta-hexabromocyclododecane OR gamma-hexabromocyclododecane OR delta-hexabromocyclododecane OR epsilon-hexabromocyclododecane)
CAS numbers:	25637-99-4, 3194-55-6, 134237-50-6, 134237-51-7, 134237-52-8, 878049-08-2, 878049-05-9, 878049-04-8, 678970-17-7, 678970-16-6, 678970-15-5, 169102-57-2, 138257-19-9, 138257-18-8, 138257-17-7
Grey literature:	- Assessments done by national and international bodies (e.g. in Google or from papers, ECHA, NTP). - Dedicated search in Organohalogen Compounds database (extended abstracts from DIOXIN conferences). To provide list in a table. - Dedicated search in the BFR conference abstracts available from its website.

Table B.2: Outcome of the literature searches

	Date	Search string	N hits
WOS (all databases)	05.02.2019	TS=(hexabromocyclododecane OR hexabromocyclododecane* OR HBCDD OR HBCDDs OR *hexabromocyclododecane OR alpha-hexabromocyclododecane OR beta-hexabromocyclododecane OR gamma-hexabromocyclododecane OR delta-hexabromocyclododecane OR epsilon-hexabromocyclododecane) AND P=(2010-2019) Refined by: [excluding] DOCUMENT TYPES: (EDITORIAL OR DATA STUDY OR LETTER OR DATA SET OR UNSPECIFIED OR DATA PAPER) Search language=English	1,002 (after automatic duplicate removal in EndNote)
PUBMED	05.02.2019	(((hexabromocyclododecane OR hexabromocyclododecane* OR HBCDD OR HBCDDs OR *hexabromocyclododecane OR alpha-hexabromocyclododecane OR beta-hexabromocyclododecane OR gamma-hexabromocyclododecane OR delta-hexabromocyclododecane OR epsilon-hexabromocyclododecane))) AND (("english"[Language]) AND ("2010"[Date - Publication] : "3000"[Date - Publication]))	613 (after automatic duplicate removal in EndNote)

	Date	Search string	N hits
SciFinder	05.02.2019	hexabromocyclododecane OR hexabromocyclododecanes (1166) 25637-99-4 (820) 3194-55-6 (70) 134237-50-6 (439) 134237-51-7 (363) 134237-52-8 (384) 878049-08-2 (1) 878049-05-9 (1) 878049-04-8 (1) 678970-17-7 (25) 678970-16-6 (18) 678970-15-5 (26) 169102-57-2 (23) 138257-19-9 (23) 138257-18-8 (20) 138257-17-7 (1) Selected on: Journal, review and book 2010–2019 English	1,211 (after automatic duplicate removal in EndNote)
ALL databases combined			1,412 (after automatic duplicate removal in DistillerSR)

Appendix C – Histological evaluation in Maranghi et al. (2013), Rasinger et al. (2014, 2018)

Table C.1: Histological evaluation of potential target tissues of HBCDDs toxicity in mice after 28 days of repeated dietary exposure to HBCDDs at daily doses of 49.5 μg/kg bw per day or 199 mg/kg bw per day (Maranghi et al., 2013; Rasinger et al., 2014, 2018). Significant effects at the $p < 0.05$ and $p < 0.01$ levels are indicated by (+) and (++), respectively

Tissue	Observations	Control	HBCDDs low dose (49.5 μg/kg bw per day)	HBCDDs high dose (199 mg/kg bw per day)
Thyroid	Desquamation into follicular lumen	0/7 (0%)	3/7 (43%)	1/7
	Foaming colloid	1/7 (14%)	2/7 (29%)	NA
Uterus	Irregular multistratification of the luminal epithelium	1/8 (12%)	2/7 (29%)	NA
	Reduction in endometrial glands density	0/8 (0%)	4/7(+) (57%)	NA
Liver	Marked vacuolisation in hepatocytes	0/10 (0%)	5/8(++) (62%)	5/8(++)
	Lymphocytic infiltration	0/10 (0%)	6/8(++) (75%)	6/8(++)
	Hyperaemic vessels	0/10 (0%)	6/8(++) (75%)	6/8(++)
	Pyknotic nuclei	0/10 (0%)	2/8 (25%)	2/8
Adrenals	Thickness external capsule	2/10 (20%)	3/7 (43%)	NA
Thymus	Hassal's bodies	2/10 (20%)	5/10 (50%)	2/9
	Cortical invasivity	2/10 (20%)	2/10 (20%)	8/9(++)
	Minerals	2/10 (20%)	5/10 (50%)	NA
	Stress	0/10 (0%)	5/10 (+) (50%)	NA
Spleen	Lymphocyte hyperplasia	4/10 (40%)	2/10 (20%)	5/10
	Infiltration in red pulp and periarteriolar zones (PALS)	0/10 (0%)	2/10 (20%)	0/10
Brain	Eosinophilic cells in the cortex	2/10 (20%)	4/9 (44%)	NA
	Eosinophilic cells in the thalamus	3/10 (30%)	4/9 (44%)	NA
	Condensed cells in the hypothalamus	0/10 (0%)	0/9 (0%)	NA

NA: not analysed.

Appendix D – Mode of action studies

Reference	Comments
Li et al. (2017b). Hexabromocyclododecane-induced Genotoxicity in Cultured Human Breast Cells through DNA Damage.	Largely observational study. HBL-100 cells were exposed to high concentrations (0, 5, 10 and 50 mg/L) for 24 h. LDH leakage observed at 5 mg/L and significant ROS and comet tail migration at 50 mg/L. Expression of transcripts for ATM, and DNA repair genes OGG1 and MTH was increased and that for tumour suppressor gene BRCA1 downregulated.
Krivoshiev et al. (2015). Elucidating toxicological mechanisms of current flame retardants using a bacterial gene profiling assay.	Unclear what concentration was used and genes < 2-fold regulated by HBCDDs.
Wielogorska et al. (2015). Endocrine disruptor activity of multiple environmental food chain contaminants.	Mammalian reporter gene assay for ER activation by β-HBCDD. No activation found.
Kovarich et al. (2011). Quantitative structure-activity relationship classification models for prediction of endocrine disrupting activity of brominated flame retardants.	γ-HBCDD predicted to be anti-oestrogenic by QSAR.
Papa et al. (2013). QSAR prediction of the competitive interaction of emerging halogenated pollutants with human transthyretin SAR QSAR.	γ-HBCDD used in training set as a BFR without T4-TTR competing potency.
Anisuzzaman and Whalen (2016). Tetrabromobisphenol A and hexabromocyclododecane alter secretion of IL-1β from human immune cells.	Human natural killer (NK) cells, monocyte-depleted (MD) peripheral blood mononuclear cells (MD-PBMC) and PBMC exposed to 0.05–5.0 mM of HBCDD for 24 h, 48 h and 6 days. Interfere with the ability of immune cells to secrete IL-1B. Exposure to HBCDD from 0.5–5.0 mM caused increases in IL-1B secretion. Inhibitors of ERK1/2 and Casp1 ameliorated HBCDD induced IL-1B release.
Anisuzzaman and Whalen (2016). Hexabromocyclododecane and tetrabromobisphenol A alter secretion of interferon gamma (IFN-gamma) from human immune cells.	Human natural killer (NK) cells, monocyte-depleted (MD) peripheral blood mononuclear cells (MD-PBMC) and PBMC exposed to 0.05–5.0 mM of HBCDD for 24 h, 48 h and 6 days. interfere with the ability of immune cells to secrete IFN-gamma. HBCDD stimulated secretion if IFN gamma but response very different in cells from different donors. Also baseline values were different.
Cato et al. (2014). Brominated flame retardants, tetrabromobisphenol A and hexabromocyclododecane, activate mitogen-activated protein kinases (MAPKs) in human natural killer cells.	HBCDDs interfere with NK-cell(s) lytic function. HBCDDs at 2.5 uM and above increased phosphorylation of p44/42; 1 uM and above increase phospho-MEK1/2. p44/42 is required for NK lysis of tumour target cells
Hinkson and Whalen (2010). Hexabromocyclododecane decreases tumor-cell-binding capacity and cell-surface protein expression of human natural killer cells.	Exposure of NK-cells to 10 μM HBCDDs for 24 h caused > 90% loss of lytic function of NK-cells and 71% decrease in NK-cell binding function, and in expression of cell surface markers CD16 (58%) and CD56 (25%). NK-cells exposed to 10 μM HBCDDs for 1 h followed by 24 h in HBCDD-free media showed 89% loss of lytic function and decreased binding function (79.2%), and CD 16 expression (48.1%).
Koike et al. (2016). Brominated flame retardants, hexabromocyclododecane and tetrabromobisphenol A, affect proinflammatory protein expression in human bronchial epithelial cells via disruption of intracellular signalling.	HBCDDs exposure (10 μg/mL) increased the expression of ICAM-1 and the production of IL-6 and -8 in human bronchial epithelial cells (BEAS-2B). HBCDDs caused activation of nuclear factor-kappa B (p50, p65) and activator protein 1 (c-Jun). HBCDDs showed no binding to nuclear oestrogen or thyroid hormone receptors.

Reference	Comments
Steves et al. (2018). Ubiquitous Flame-Retardant Toxicants Impair Spermatogenesis in a Human Stem Cell Model.	Human stem cell-based model of spermatogenesis, indicating that HBCDDs affect spermatogonia and primary spermatocytes through mitochondrial membrane potential perturbation and ROS generation, resulting in apoptosis. HBCDD concentrations: 1 uM, 10 uM, 25 uM, 50 uM, 100 uM and 200 μM dissolved in dimethyl sulfoxide (DMSO). Reduced viability at 25 μM but still 80% viability at 200 μM. Loss of marker (PLZF) for stem and progenitor spermatogonia observed at 1 μM. Primary spermatocyte marker piwi like RNA-mediated gene silencing 2 (HILI) increased in expression from 1 to 25 μM and then declined.
Yasmin and Whalen (2018). Flame retardants, hexabromocyclododecane (HCBD) and tetrabromobisphenol a (TBBPA), alter secretion of tumor necrosis factor alpha (TNFα) from human immune cells.	Effects of HBCDDs on secretion of tumour necrosis factor alpha (TNFα) from human immune cells. In presence of T-cells, HBCDDs increased TNFα secretion, but without T-cells HBCDD caused reduction. HBCDD-induced increases in TNFα secretion utilised the p38 MARK pathway.
Zhang et al. (2015). Transcriptomic and metabolomic approaches to investigate the molecular responses of human cell lines exposed to the flame retardant hexabromocyclododecane (HBCD).	Transcriptomic and metabolomic effects of HBCDDs on A549 and HepG2/C3A cells. MTT assay for A549 and HepG2 cells indicated EC50 values of 27.4 μM and 63.0 μM, respectively. Little effect on gene expression or metabolome in either cell type up to the highest dose of 4 μM.
Zou et al. (2013). PI3K/Akt pathway mediates Nrf2/ARE activation in human L02 hepatocytes exposed to low-concentration HBCDs.	Nanomolar concentrations of HBCDDs stimulate L02 cell proliferation in a DNA-PKcs-dependent manner, increase protein levels and nuclear translocation of Nrf2, leading to upregulation of its target gene HO-1. The PI3K/Akt pathway is essential for HBCDD activation of the Nrf2-ARE pathway L02 cells.
Dorosh et al. (2010). Assessing oestrogenic effects of brominated flame retardants hexabromocyclododecane and tetrabromobisphenol A on MCF-7 cells.	E-screen assay in MCF-7 cells. HBCDDs increased cell proliferation and dose-dependently increased gene expression of TFF1 with effects starting at 20–200 nM. Blocked by the anti-oestrogen, ICI 182,780.
Arini et al. (2017). A cell-free testing platform to screen chemicals of potential neurotoxic concern across twenty vertebrate species.	High throughput cell-free neurochemical assay based on receptors and enzymes. Not much specific information on HBCDDs.
An et al. (2014a). Hexabromocyclododecane and polychlorinated biphenyls increase resistance of hepatocellular carcinoma cells to cisplatin through the phosphatidylinositol 3-kinase/protein kinase B pathway.	HBCDDs reduces sensitivity of HCC cells (HepG2, MHCC97H and MHCC97L) to cisplatin through modulation of the NF-kB pathway activation and p53 and associated with the PI3K/Akt pathway activity.
Al-Mousa and Michelangeli (2014). The sarcoplasmic-endoplasmic reticulum Ca(2+)-ATPase (SERCA) is the likely molecular target for the acute toxicity of the brominated flame retardant hexabromocyclododecane (HBCD).	Six BFRs assessed for cytotoxicity and potency to inhibit SERCA. Strong Direct correlation (r=0.94) between the potencies of inducing cell death and inhibiting SERCA. Ki of HBCDDs for SERCA was 2.7 uM. Mechanistic studies indicate that HBCDDs prevent ATP binding to SERCA.
Al-Mousa and Michelangeli (2012). Some commonly used brominated flame retardants cause Ca2+-ATPase inhibition, beta-amyloid peptide release and apoptosis in SH-SY5Y neuronal cells.	HBCDDs caused dose-dependent increase in $[Ca2+]_i$ and inhibits the SERCA with an apparent Ki of 3.5 μM in SH-SY5Y human neuroblastoma cells. Increase in ROS formation, cytochrome C release and apoptosis.
Bastos Sales et al. (2013). Effects of endocrine disrupting chemicals on in vitro global DNA methylation and adipocyte differentiation.	No effect of 10 μM HBCDDs on DNA methylation and adipocyte differentiation.
Christen et al. (2010). Some flame retardants and the antimicrobials triclosan and triclocarban enhance the androgenic activity in vitro.	Studied androgenic and antiandrogenic activity of BFRs and antimicrobials *in vitro* in MDA-kb2 cells. HBCDDs very low activity but enhances androgenic activity.

Reference	Comments
Fa et al. (2013). Acute effects of hexabromocyclododecane on Leydig cell cyclic nucleotide signaling and steroidogenesis in vitro.	HBCDDs inhibited basal and hCG-stimulated cAMP production, but elevated basal steroidogenesis. HBCDDs decrease in mitochondrial membrane potential in untreated and hCG-treated cells.
Fa et al. (2015). HBCDD-induced sustained reduction in mitochondrial membrane potential, ATP and steroidogenesis in peripubertal rat Leydig cells.	HBCDDs caused a sustained reduction in ATP level. Accumulation of cAMP and androgen were also reduced. There was inhibition in the expression of genes for steroidogenic enzymes, luteinising hormone receptor, regulatory and transport proteins and a decrease in abundance of StAR. Enzymatic experiments indicated loss of activities of (CYP11A1) and 17β-hydroxysteroid dehydrogenase (HSD17β).
Fa et al. (2014). Hexabromocyclododecane facilitates FSH activation of ERK1/2 and AKT through epidermal growth factor receptor in rat granulosa cells.	HBCDDs potentiate FSH-stimulated phosphorylation of EGFR, ERK1/2 and AKT, indicating a direct effect on EGFR.
Huang et al. (2016b). In vitro study on the biotransformation and cytotoxicity of three hexabromocyclododecane diastereoisomers in liver cells.	Cytotoxicity of HBCDDs in L02 and HepG2 cells was β-HBCDD > γ-HBCDD > α-HBCDD.
Ibhazehiebo et al. (2011a). 1, 2, 5, 6, 9, 10-αHexabromocyclododecane (HBCD) impairs thyroid hormone-induced dendrite arborisation of Purkinje cells and suppresses thyroid hormone receptor-mediated transcription.	HBCDDs at 0.1 nM suppressed TR-mediated transcription in a reporter gene assay, and suppressed TH-induced dendrite arborisation of Purkinje cells in primary cerebellar culture derived from rat neonates.
Ibhazehiebo et al. (2011b). Brain-derived neurotrophic factor (BDNF) ameliorates the suppression of thyroid hormone-induced granule cell neurite extension by hexabromocyclododecane (HBCD).	HBCDDs at 0.1 nM suppressed TH-induced neurite extension of granule cell aggregate in primary rat cerebellar granule cell aggregate cultures. BDNF ameliorated this effect in presence of T3. Results indicate that T3-stimulated increase in BDNF may be involved in HBCDD-induced impairment of TH-mediated neuritogenesis of granule cells.
Kim et al., 2016. Influence of hexabromocyclododecane and 4-nonylphenol on the regulation of cell growth, apoptosis and migration in prostatic cancer cells *Toxicol In Vitro*, 32: 240-7.	HBCDDs may enhance progression of prostate cancer by modulating growth and migration of LNCaP prostate cells by acting on cell cycle and apoptosis.
Koike et al. (2013). Brominated flame retardants stimulate mouse immune cells in vitro. *J Appl Toxicol*, 33(12): 1451-9.	HBCDDs increased expression of T-cell receptor, MHC class II and CD86 as well as IL-4 production in splenocytes. Authors suggested that HBCDDs may induce or enhance immune/ allergic responses by increasing antigen presentation-related molecule expression and IL-4 production.
Krivoshiev et al. (2016). Assessing in-vitro estrogenic effects of currently-used flame retardants.	HBCDDs stimulated proliferation of MCF-7 cells with an EC20 of 5.5 μM. HBCDDs also inhibited 17B-oestradiol stimulated proliferation with an IC20 of 17.6 μM.
Park et al. (2012). Cell growth of BG-1 ovarian cancer cells is promoted by di-n-butyl phthalate and hexabromocyclododecane via upregulation of the cyclin D and cyclin-dependent kinase-4 genes.	0.2 μM HBCDDs stimulated proliferation of BG-2 ovarian cancer cells. HBCDDs upregulated mRNA for cyclin D and cyclin-dependent kinase-4 (cdk-4), which are downstream target genes of ER.

Reference	Comments
Reffatto et al. (2018). Parallel in vivo and in vitro transcriptomics analysis reveals calcium and zinc signalling in the brain as sensitive targets of HBCD neurotoxicity.	Neurotoxic potential of HBCDDs was studied in mouse brain and, to separate direct effects from system responses (e.g. via hormones) two cell lines of mouse neuronal origin, NSC-19 and N2A, as well as in primary hippocampal neuronal cultures. Transcriptome profiling of mouse brains and the two cell lines indicated that sex steroid regulation, and Ca^{2+} and Zn^{2+} homeostasis in glutamatergic neurons were preferentially affected. Follow-up experiments on the two cell lines and isolated primary hippocampal neurons confirmed effects of HBCDDs on free [Zn^{2+}], glutamate-induced post-synaptic [Ca^{2+}] and zinc-stimulated post-synaptic [Ca^{2+}] release, which was almost completely blocked by 1 uM HBCDDs. The increase in cytosolic free [Zn^{2+}] could be partially blocked by an antioxidant, indicating ROS formation as a contributing factor.
Saegusa et al. (2012). Transient aberration of neuronal development in the hippocampal dentate gyrus after developmental exposure to brominated flame retardants in rats.	Female pregnant SD rats were fed a diet containing 0 (control), 100, 1,000 or 10,000 mg/L of HBCDDs from GD 10 to PND20. Effects on neuronal development was observed at 1,000 and 10,000 mg/L. $Reelin^{+}$ and $NeuN^{+}$ cells in interneurons of the dentate hilus increased temporarily, suggestive of aberrant neuronal migration. Effect on $reelin^{+}$ cells was only seen at 1,000 mg/L (not at 10,000 mg/L). An increase in apoptotic bodies was observed at PND20 in the subgranular zone. At PND20 there was a small reduction in serum T3.
Suzuki et al. (2013). Similarities in the endocrine-disrupting potencies of indoor dust and flame retardants by using human osteosarcoma (U2OS) cell-based reporter gene assays.	CALUX reporter gene assays, based on U2OS cells were used to evaluate reporter gene assays used to evaluate activities of flame retardants on the human androgen receptor (AR), oestrogen receptor α (ERα), progesterone receptor (PR), glucocorticoid receptor (GR) and peroxisome proliferator-activated receptor γ2 (PPARγ2). HBCDD showed relatively mild antagonistic effect on the AR and PR receptors.
Wilson et al. (2016). Do persistent organic pollutants interact with the stress response? Individual compounds, and their mixtures, interaction with the glucocorticoid receptor.	HBCDDs were not tested alone but in chemical mixture.
Wu et al. (2016b). Hexabromocyclododecane exposure induces cardiac hypertrophy and arrhythmia by inhibiting miR-1 expression via up-regulation of the homeobox gene Nkx2.5.	Zebrafish and H9C2 rat cardiomyocyte cells. Zebrafish embryos were exposed to 0, 2, 20 and 200 nM HBCDD in the water. Exposure to 20 and 200 nM resulted in cardiac hypertrophy and increased deposition of collagen. miR-1 might mediate HBCDD induced cardiac hypertrophy and arrhythmia via its target genes Mef2a and Irx5. H9C2 rat cardiomyocyte cells exposed to HBCDDs showed reduced Ca^{2+}-ATPase activity and elevated Ca^{2+} in the cytosol.
Zhang et al. (2016). Gene expression and metabolic responses of HepG2/C3A cells exposed to flame retardants and dust extracts at concentrations relevant to indoor environmental exposures.	Multiomics approach used to interrogate effects of dust extract and BFR mixture (including HBCDDs) in HepG2 cells. Exposure of cells to dust extracts induced expression of several CYP biotransformation enzymes. Such effects were not observed in the mixture of BFRs and it was concluded that other components of the dust extract were likely causing the observed effects.

Reference	Comments
Zhong et al. (2015). HBCD and PCBs enhance the cell migration and invasion of HepG2 via the PI3 K/Akt pathway.	HepG2 cells exposed to HBCDDs were assessed for cell viability, apoptosis, cell migration and invasion using cell counting kit-8 (CCK-8) assay, flow cytometry, cell scratch assay, respectively. 10 nM HBCDDs stimulated migration and invasion of HepG2 cells, increased protein expression of MMP9 and suppressed E-cadherin (CDH1) expression. HBCDD exposure increased stimulatory phosphorulation of protein kinase B and extracellular signal-regulated kinase 32 (ERK), and expression of mammalian target of rapamycin (mTOR).
Ziemiliska et al. (2012). Acute cytotoxicity evoked by tetrabromobisphenol A in primary cultures of rat cerebellar granule cells outweighs the effects of polychlorinated biphenyls.	Mild effect of 25 μM HBCDDs on calcium uptake in CGCs.
Zimmer et al. (2011). In vitro steroidogenic effects of mixtures of persistent organic pollutants (POPs) extracted from burbot (Lota lota) caught in two Norwegian lakes.	HBCDDs were not tested alone but in chemical mixture.
An et al. (2013). The cytological effects of HBCDs on human hepatocyte L02 and the potential molecular mechanism.	High concentration of HBCDDs (> 20 μM) decreased survival of L02 hepatocytes, lower dose of HBCDDs (10^{-13}–10^{-7} M) stimulated cell proliferation and up-regulation of PCNA protein expression level.
An et al. (2016). The 'adaptive responses' of low concentrations of HBCD in L02 cells and the underlying molecular mechanisms.	L02 cells were able to acquire tolerance to a high concentration of α-HBCDD through pre-exposure to a lower dose. They provided evidence that the mechanism of this is through activation of the PI3K/Akt pathway, reduced phosphorylation of AMPK and increased phosphorylation of p38 MAPK.
An et al. (2014b). Oligomeric proanthocyanidins alleviate hexabromocyclododecane-induced cytotoxicity in HepG2 cells through regulation on ROS formation and mitochondrial pathway.	This study examined effects of oligomeric proanthocyanidins (OPCs) on cytotoxicity induced by HBCDDs. Whilst interesting it is not considered relevant to the RA of HBCDDs.
Canbaz et al. (2016). Indoor pollutant hexabromocyclododecane enhances house dust mite-induced activation of human monocyte-derived dendritic cells.	Simultaneous exposure of monocyte dendritic cells to house mite dust allergens and HBCDDs, and HBCDDs increased the expression of HLA-DR, co-stimulatory molecule CD86 and pro-inflammatory cytokine IL-8 depending on the dose of HBCDDs (1–20 μM). An increase in IL-8 was observed at 1 μM but other effects required higher concentrations (10–20 μM).
Wang et al. (2016a). New Insights into the Cytotoxic Mechanism of Hexabromocyclododecane from a Metabolomic Approach.	Detailed study on HepG2 cells, starting with NMR and following up with hypothesis driven experiments and analyses. HepG2 cells exposed to reagent-grade HBCDD formula (purity: > 95%, Aladdin Industrial Corp.) at 0.05, 1 and 10 mg/L. HBCDD exposure resulted in amino acid metabolism, protein biosynthesis, fatty acid metabolism and phospholipid metabolism. Beta oxidation of long-chain fatty acids was suppressed, ATP production reduced, Na/K-ATPase inhibited and uptake of amino acids and glucose impaired. Reduced beta oxidation was accompanied by increase in free fatty acids and increased synthesis of phospholipids. The authors suggest that most effects may be a results of reduced ATP production and consequential loss of Na/K-ATPase and Ca-ATPase activities, leading to reduced glucose and amino acid uptake by cells.
Georgantzopoulou et al. (2014). P-gp efflux pump inhibition potential of common environmental contaminants determined in vitro.	The calcein-acetoxymethyl ester (calcein-AM) assay in P-glycoprotein–overexpressing Madin–Darby canine kidney cells (MDCKII–MDR1) was exploited to study the inhibition of P-gp efflux pumps by HBCDDs (technical mixture) and other aquatic contaminants. Data suggested that HBCDDs may stimulate the P-gp efflux pump.

Reference	Comments
Kamata et al. (2018). Agonistic effects of diverse xenobiotics on the constitutive androstane receptor as detected in a recombinant yeast-cell assay	HBCDDs have a relatively low potential to activate CAR in a recombinant yeast assay.
Lille-Langoey et al. (2015). Environmental contaminants activate human and polar bear (*Ursus maritimus*) pregnane X receptors (PXR, NR1I2) differently.	COS-7 cells transfected with luciferase reporter gene construct was used to assess activation of polar bear and human PXR. A technical HBCDD mixture containing 81% γ-HBCDD was tested. The HBCDD mixture had a moderate potency to activate PXR compared with other chemicals tested and maximum activation was 41% of that obtained by rifampicin exposure.
Montano et al. (2011). Effects of mixtures of persistent organic pollutants (POPs) derived from cod liver oil on H295R steroidogenesis.	HBCDDs were not tested alone but in chemical mixture.
van den Dungen et al. (2017). Persistent organic pollutants alter DNA methylation during human adipocyte differentiation.	DNA methylation and gene expression were studied in response to exposure to different POPs during human adipocyte differentiation. HBCDDs did not affect lipid accumulation in adipocytes. 1 μM HBCDD induced ALP activity in adipocytes.
van den Dungen et al. (2015). Steroid hormone related effects of marine persistent organic pollutants in human H295R adrenocortical carcinoma cells.	Effects of various POPs and mixtures thereof were studied in H295R adrenocortical carcinoma cells in relation to endocrine effects. HBCDDs did not affect any steroid hormone levels. 100 μM HBCDDs induced expression of CYP19A1.
Kang et al. (2012). Induced growth of BG-1 ovarian cancer cells by 17beta-estradiol or various endocrine disrupting chemicals was reversed by resveratrol via downregulation of cell cycle progression.	Treatment of BG-1 cells with HBCDDs resulted in an increase of cell growth.
Li et al. (2017c). Neuroprotection by Taurine on HBCD-Induced Apoptosis in PC12.	Exposure of PC12 cells to 10 μM HBCDDs reduced protein expression of Bcl-2, increased expression in Bax protein and activity of caspase-3. Taurine protected against these effects.
Liu et al. (2017). Taurine Alleviate Hexabromocyclododecane-Induced Cytotoxicity in PC12 Cells via Inhibiting Oxidative Stress.	Taurine protected against cytotoxicity induced by HBCDDs in PC12 cells through inhibition of oxidative stress.

Annex A – Protocol for the risk assessments for human health related to the presence of brominated flame retardants (BFRs) in food

The Annex is provided as a separate pdf file containing the risk assessment protocol selected by the CONTAM Panel to update the previous risk assessments of brominated flame retardants (BFRs) in food and is available on the EFSA Knowledge Junction community on Zenodo at: https://doi.org/10.5281/zenodo.4475651

Annex B – Occurrence data on HBCDDs in food submitted to EFSA and dietary surveys per country and age group available in the EFSA Comprehensive Database, considered in the exposure assessment

The Annex is provided as a separate Excel file containing the occurrence data submitted to EFSA and dietary surveys per country and age group is available on the EFSA Knowledge Junction community on Zenodo at: https://doi.org/10.5281/zenodo.4475651

At this link, the raw occurrence dataset on HBCDDs as extracted from the EFSA data warehouse is available.

Annex C – Benchmark dose analysis

The Annex is provided as a separate pdf file containing the results of the benchmark dose (BMD) analysis from which no reference point was selected and is available on the EFSA Knowledge Junction community on Zenodo at: https://doi.org/10.5281/zenodo.4475651

Annex D – Chronic dietary exposure to HBCDDs and the contribution of different food groups to the dietary exposure

The Annex is provided as a separate excel file containing the chronic dietary exposure assessment to HBCDDs per survey and the contribution of different food groups to the dietary exposure and is available on the EFSA Knowledge Junction community on Zenodo at: https://doi.org/10.5281/zenodo.4475651

Annex E – Outcome of the Public consultation on the Update of the risk assessment of hexabromocyclododecanes (HBCDDs) in food

The Annex is provided as a separate pdf file contain the outcome of the public consultation of the draft scientific Opinion, including the comments received and how they were taken into account when finalising the scientific Opinion, and is available on the EFSA Knowledge Juntion community on Zenodo at: https://doi.org/10.5281/zenodo.4475651