Substance Name: Bis(pentabromophenyl) ether

EC Number: 214-604-9

CAS Number: 1163-19-5

MEMBER STATE COMMITTEE

SUPPORT DOCUMENT FOR IDENTIFICATION OF BIS(PENTABROMOPHENYL) ETHER AS A SUBSTANCE OF VERY HIGH CONCERN BECAUSE OF ITS PBT/vPvB PROPERTIES

Adopted on 29 November 2012

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TABLES

Substance Name(s): Bis(pentabromophenyl) ether [DecaBDE]

EC Number(s): 214-604-9

CAS number(s): 1163-19-5

Bis(pentabromophenyl) ether is identified as PBT according to Article 57 (d) and as vPvB according to Article 57 (e).

Summary of how the substance meets the PBT or vPvB criteria

DecaBDE is widely detected in the European environment, residing mainly in sediments and soils at concentrations up to several milligrams per kilogram (parts per million) on a dry weight basis. It is also present in many types of aquatic and terrestrial wildlife species (including tissues of sensitive life stages such as bird eggs) at numerous geographical locations; although tissue concentrations are often low (close to the limits of analytical detection, or below), it can attain concentrations up to a few hundred micrograms per kilogram (parts per billion) on a wet weight basis in some top predators.

Primary degradation half-lives in sediment and soil significantly exceed 180 days, indicating that decaBDE is 'very persistent' according to the Annex XIII criteria. On the basis of the available data, it can also be concluded that there is a high probability that decaBDE is transformed in soil and sediments to form substances which either have PBT/vPvB properties, or act as precursors to substances with PBT/vPvB properties, in individual amounts greater than 0.1% over timescales of a year. Transformation to such substances within biota provides an additional pathway for the exposure of organisms. High persistence combined with wide distribution in the environment creates a high potential for lifetime exposure and uptake in organisms, and a pool of the substance in many localities that will act as a long-term source of degradation products through both abiotic and biotic transformation.

On the basis of all of the evidence that is now available, decaBDE is considered to meet the definition of a PBT/vPvB substance in accordance with Annex XIII of the REACH Regulation, and thereby fulfils the criteria in Articles 57(d) and (e).

Registration dossiers submitted for this substance: Yes

Justification

1 Identity of the substance and physical and chemical properties

1.1 Name and other identifiers of the substance

Table 1: Substance identity

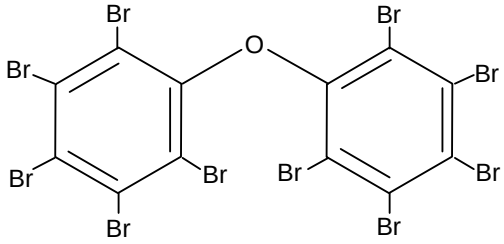
EC number:	214-604-9
EC name:	Bis(pentabromophenyl) ether
CAS number (in the EC inventory):	1163-19-5
CAS numbers:	1163-19-5, 109945-70-2, 145538-74-5, 1201677-32-8
CAS name:	Benzene, 1,1'-oxybis[2,3,4,5,6-pentabromo-]
IUPAC name:	1,1'-Oxybis(pentabromobenzene)[1]
Index number in Annex VI of the CLP Regulation	Not applicable
Molecular formula:	$C_{12}Br_{10}O$
Molecular weight:	959.2 g/mole
Synonyms:	decabromodiphenyl ether; decabromodiphenyl oxide; bis(pentabromophenyl) oxide; decabromo biphenyl oxide; decabromo phenoxybenzene; benzene 1,1' oxybis-, decabromo derivative; decaBDE; DBDPE; DBBE; DBBO; DBDPO

Note: The abbreviation decaBDE is used throughout this document to refer to the substance for brevity[2]. The molecule is also known by a specific congener number (BDE-209) in the IUPAC PBDE nomenclature system (Appendix 2 provides a full list of these). This congener number is referred to where comparison with the other congeners is important.

[1] The IUPAC name disseminated with registration information is different to the IUPAC names notified in the Classification and Labelling (C+L) inventory. The following IUPAC names have been notified to the C+L inventory: 2,3,4,5,6-pentabromo-1-(2,3,4,5,6-pentabromophenoxy) benzene, bis(pentabromophenyl) ether, decabromodiphenyl ether, decabromdiphenylether, decabromodiphenyl oxide.

[2] Individual groups of PBDE congeners are also referred to in abbreviated form (e.g. hexaBDE for hexabromodiphenyl ethers).

Structural formula:



1.2 Composition of the substance

Name: bis(pentabromophenyl) ether

Degree of purity: The composition of the commercial product from different manufacturers/importers is marked as confidential in the Chemical Safety Reports. However, it is generally consistent with the information given in Tables 2 and 3 (as reported in the original ESR assessment).

Table 2: Constituents in the currently supplied commercial substance

Constituents	Typical concentration	Concentration range	Remarks
Decabromodiphenyl ether	97.4%	97-98%	EC, 2002

Table 3: Impurities

Impurities	Typical concentration	Concentration range	Remarks
Nonabromodiphenyl ether	2.5%	0.3-3%	EC, 2002
Octabromodiphenyl ether	0.04%	Not known	

The individual congener groups may consist of more than one isomer. For example, Timmons and Brown (1988) detected three nonaBDE and three octaBDE congeners in a commercial decaBDE product using a high resolution gas chromatography – mass spectrometry (GC-MS) method. Trace amounts of other compounds, thought to be hydroxybrominated diphenyl compounds were also tentatively identified as impurities. EC (2002) also indicated that lower molecular weight PBDE congeners may be present at concentrations up to 0.005% w/w. This finding was supported by Hamm et al. (2001), who performed a trace analysis of a composite sample of commercial decaBDE from three suppliers. Total tri-, tetra-, penta-, hexa- and heptaBDEs were each present at concentrations below 0.0039 % w/w.

The composition of older products or products from other sources may be different. For example, a product that is no longer supplied in the EU had a composition of 77.4% decaBDE, 21.8% nonaBDE and 0.85% octaBDE (EC, 2002).

There are no stated additives incorporated into the commercially available forms of this substance (EC, 2002).

1.3 Name and other identifiers of transformation products

The principal transformation products that are the focus of this report are listed in Table 4.

Table 4: Identifiers for PBDE congeners

	TetraBDE	**PentaBDE**	**HexaBDE**	**HeptaBDE**	**OctaBDE**	**NonaBDE**
EC number:	254-787-2	251-084-2	253-058-6	273-031-2	251-087-9	264-565-7
EC name:	diphenyl ether, tetrabromo derivative	diphenyl ether, pentabromo derivative	diphenyl ether, hexabromo derivative	diphenyl ether, heptabromo derivative	diphenyl ether, octabromo derivative	pentabromo(tetrabromophenoxy)benzene
CAS number (in the EC inventory):	40088-47-9	32534-81-9	36483-60-0	68928-80-3	32536-52-0	63936-56-1
CAS number:	40088-47-9	32534-81-9	36483-60-0	68928-80-3	32536-52-0	63936-56-1
CAS name:	Benzene,1,1'-oxybis-, tetrabromo derivative	Benzene,1,1'-oxybis-, pentabromo derivative	Benzene,1,1'-oxybis-, hexabromo derivative	Benzene,1,1'-oxybis-, heptabromo derivative	Benzene,1,1'-oxybis-, octabromo derivative	Benzene,1,2,3,4,5-pentabromo-6-(tetrabromophenoxy) derivative
Index number in Annex VI of the CLP Regulation	-	602-083-00-4	-	-	602-094-00-4	-
Molecular formula:	$C_{12}H_6Br_4O$	$C_{12}H_5Br_5O$	$C_{12}H_4Br_6O$	$C_{12}H_3Br_7O$	$C_{12}H_2Br_8O$	$C_{12}HBr_9O$
Molecular weight:	485.82	564.72	643.62	722.48	801.42	880.27

1.4 Physico-chemical properties

The data in Table 5 are taken from the original ESR assessment (EC, 2002). Unless otherwise stated, they are also cited in the registration dossiers for the substance (as summarised on the ECHA website[3]). No new literature search has been conducted for physico-chemical data.

Table 5: Overview of relevant physicochemical properties

REACH ref Annex, §	Property	Value	Reference
V, 5.1	Physical state at 20°C and 101.3 kPa	Fine, white to off-white crystalline powder	EC (2002)
V, 5.2	Melting/freezing point	300-310°C	Dead Sea Bromine Group, 1993, cited in EC (2002)
V, 5.3	Boiling point	Decomposes at >320°C	Dead Sea Bromine Group, 1993, cited in EC (2002)
V, 5.5	Vapour pressure	4.63×10^{-6} Pa at 21°C	Wildlife International Ltd, 1997, cited in EC (2002)
V, 5.7	Water solubility	<0.1 µg/l at 25°C (column elution method)	Stenzel and Markley, 1997, cited in EC (2002)
V, 5.8	n-Octanol/water partition coefficient, K_{ow} (log value)	6.27 (measured – generator column method) 9.97 (estimated using an HPLC method) [a]	MacGregor & Nixon, 1997, and Watanabe & Tatsukawa, 1990, respectively, cited in EC (2002)
VII, 5.19	Dissociation constant (pKa)	Not relevant	-

Note: a – Not included in the registration dossiers.

From the above table only the EC (2002) reference has been included in the reference list; those cited from within the EC (2002) reference have not been referenced.

2 Harmonised classification and labelling

DecaBDE is not listed in Annex VI of Regulation (EC) No. 1272/2008 (the CLP Regulation). The 2nd ATP to the CLP Regulation introduced additional classification criteria based on long-term aquatic hazard data. However, these do not affect the classification of the substance in view of the lack of any observed chronic toxicity in standard aquatic tests up to water solubility limit.[4]

[3] http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances.

[4] As discussed in Section 5, recent studies suggest some effects on fish and amphibians exposed to decaBDE at or around the water solubility limit or via the diet over long-term exposures. The difficulty in maintaining test concentrations and non-standard methods imply that these studies should be repeated using standardised test guidelines before a decision can be taken about the reliability of the observations. However, they suggest that chronic aquatic classification may be warranted.

The following self-classifications have been notified to the CLP Inventory as of 8 November 2012 (no specific concentration limits have been proposed and no notes applied):

Classification				Number of Notifiers	Joint Entries
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Pictograms Signal Word Code(s)**		
Not Classified				163	√
Acute Tox. 4	H302	H302	GHS07 Wng	28	
Acute Tox. 4	H312	H312			
Eye Irrit. 2	H319	H319			
Acute Tox. 4	H302	H302	GHS07 Wng	23	
Acute Tox. 4	H312	H312			
Acute Tox. 4	H332	H332			
Muta. 2	H341	H341	GHS08 Wng	14	
STOT RE 2	H373	H373			
Aquatic Chronic 4	H413	H413		12	
Aquatic Chronic 4	H413	H413		7	
Classification				**Number of Notifiers**	**Joint Entries**
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Pictograms Signal Word Code(s)**		
		H413	GHS07 GHS05 GHS08	2	
		H351			

Classification				Number of Notifiers	Joint Entries
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Pictograms Signal Word Code(s)**		
		H318	Dgr		
		H302			
Eye Irrit. 2	H319	H319	GHS08 Dgr	1	
Muta. 2	H341	H341			
Acute Tox. 4	H302	H302	GHS07 Wng	1	
Eye Irrit. 2	H319	H319			
Aquatic Chronic 4	H413	H413			
		H312		1	
Aquatic Chronic 4	H413				
Aquatic Chronic 4	H413			1	

Number of Aggregated Notifications: 11

The REACH registrants do not classify the substance (for health or environment). The basis for the CLP Inventory entries is unknown – they could be based on different interpretations of the same data, different data sets, or made in error. This has not been investigated further.

3 Environmental fate properties

Since the focus of this report is the transformation of decaBDE to more hazardous substances, the following sections present the greatest detail for studies that address this issue. Studies that consider decaBDE alone are only briefly covered. The discussion includes all the relevant studies that are cited in the registration dossiers (as summarised on the ECHA website), and many more. Where the interpretation of a study differs between the registration dossier and this report, this is mentioned.

Relevant fate data relating to persistence and bioaccumulation for the other PBDE congeners are summarized in Appendix 1.

3.1 Degradation

3.1.1 Abiotic degradation

3.1.1.1 Hydrolysis

The registration dossiers do not provide any information on this end point.

A standard test guideline study is not available. Gallet et al. (2001) [ABST] reported that no major degradation products were found when 5 mg of decaBDE was placed in sealed vials containing 15 ml of water at pH 5 or pH 7 for six weeks at 100°C. Standard test guidelines include pH 9, so this is an omission. However, decaBDE has a very low water solubility (<0.1 µg/l at 25 °C) and the molecule does not contain any functional groups that are readily susceptible to hydrolysis.

Hydrolysis is therefore unlikely to be a relevant degradation process in the environment.

3.1.1.2 Phototransformation/photolysis

3.1.1.2.1 Phototransformation in air

The registration dossiers do not provide any information on this end point.

A second order rate constant for the reaction of vapour phase decaBDE with atmospheric hydroxyl radicals has been calculated as 1.7×10^{-13} cm^3 $molecule^{-1}$ s^{-1} from the chemical structure using the Syracuse Research Corporation AOP program; assuming a hydroxyl radical concentration of 5×10^5 molecule/cm^3, the estimated atmospheric half-life is 94 days (EC, 2002). Since the substance has a very low vapour pressure, this is unlikely to be a significant removal pathway in the environment.

In the atmospheric compartment, decaBDE will almost exclusively be adsorbed to particulates (see Section 3.2). A large number of studies have investigated the potential for photodegradation of decaBDE on solid matrices in air, and details of the studies most relevant to this dossier are given below.

a) Palm et al. (2003) investigated the photochemical degradation of decaBDE (and certain other PBDEs) in an aerosol smog chamber. In these experiments, the test substance (along with mirex, a chlorinated hydrocarbon) was adsorbed on to silicon dioxide (specific surface area 380 m^2/g) at a concentration of around 1% w/w (this was reported to give a sub-monolayer thickness covering on the aerosol particles). The particles were then suspended in water, atomised, dried and dispersed as an aerosol in a smog chamber to give an aerosol density of around 2 mg/m^3 after 1 hour. Silicon dioxide particles were chosen because they are transparent to light, can be coated with a monolayer of molecules and allow a stable aerosol to be generated and maintained. Their size (diameter approximately 1 µm in the aerosol) was also representative of particulates found in the atmosphere. The conditions used were therefore assumed to have maximised the photolytic potential.

   The smog chamber had a large volume (1,760 litres), which enabled residence times of around 10 hours to be obtained for the aerosol particles. The aerosol was exposed to simulated sunlight (fluorescent lamps) and/or hydroxyl radicals. The hydroxyl radicals were generated either by the reaction of ozone with hydrazine in darkness or by the photochemical degradation of methyl nitrite. The hydrocarbons *n*-butane, 2,2-dimethylbutane or cyclohexane and toluene (at 30-100 ppb), and an inert standard (perfluorohexane at 60 ppb) were also added to the chamber. The rate of

degradation of decaBDE was found to be barely measurable with the equipment used and the rate constant was determined to be $<6\times10^{-13}$ cm^3 $molecule^{-1}$ s^{-1} for reaction with hydroxyl radicals.

Further experiments carried out with aerosol-borne decaBDE showed that the substance was subject to photodegradation but the rate was lower than found in an organic solvent solution (i.e. a half-life between 1.9 and 26 minutes) by at least an order of magnitude, and was also lower than the rate found in experiments using an aqueous silicon dioxide suspension (described separately in Section 3.1.1.2.2).[5]

Overall, this was a well-conducted study, which provides no evidence of significant transformation of decaBDE on particulate aerosols over 10 hours when exposed to light under the conditions of the test. The influence of co-exposure with voltatile hydrocarbons and mirex on this result is unknown.

b) Stapleton and Dodder (2006 [ABST] and 2008) investigated the photodegradation of decaBDE on household dust by natural sunlight. (Details of this study were included in ECB (2007a) based on the 2006 abstract but the Stapleton and Dodder (2008) paper is peer-reviewed and presents further details on the possible degradation products; it has not been summarised in previous EU risk assessment reports.) The dust used in the study was an indoor dust standard reference material. This was a well characterised, homogeneous material prepared from vacuum cleaner bag contents collected from homes, motels and hotels in several states in the United States. The material had certified concentrations for up to fifteen PBDE congeners.

PBDEs present in the dust were firstly removed by soxhlet extraction. Analysis of the extracted dust confirmed that concentrations of all the PBDEs were below the limit of detection (<0.2 µg/kg). The cleaned dust was then spiked with a solution of decaBDE in toluene, followed by solvent evaporation, to give a concentration of 2,180 µg/kg dry weight (dw) in the dust. In addition, a sample of standard reference dust was also used as received (this contained several PBDE congeners including decaBDE at a concentration of 2.7 mg/kg; no further decaBDE was added to this sample). The dust samples (0.5 g aliquots in UV cuvettes) were were placed on a tray lined with aluminium foil and exposed to sunlight outdoors at Gaithersburg, Maryland, United States (39º08' N, 77º13' W) between 9 a.m. and 4 p.m. Monday to Friday on days on which no precipitation was forecast for a total of up to 200 hours' exposure. Each experiment was carried out in triplicate. Three control samples were also run for each dust type (wrapped in foil and maintained at room temperature in the laboratory). The solar irradiance and temperature were determined at hourly intervals during the study. The average incident solar radiation during the experiment was 545 W/m^2 (range 61 to 929 W/m^2).

At the start of the experiment the spiked dust sample contained detectable amounts of nonaBDEs (BDE-206, BDE-207 and BDE-208; accounting for around 3.8% of the total PBDE concentration) and a trace of an unidentified heptaBDE, as well as decaBDE. The reference dust contained a range of PBDE congeners from tri- to decaBDE. The concentration of decaBDE was found to decrease with time in both sample types following exposure to sunlight. The first order removal rate constant was estimated to be 2.3×10^{-3} $hour^{-1}$ (equivalent to a half-life of 301 sunlight hours) in the spiked dust and 1.7×10^{-3} $hour^{-1}$ (equivalent to a half-life of 408 sunlight hours) in the standard reference material (note that these are extrapolated since they exceed the duration of the experiment). The authors considered that the half-life of decaBDE in indoor dust would be considerably longer than these values since dust

[5] The photolysis of aerosol-borne 2,2',4,4',5,5'-hexaBDE was also investigated. Some lower molecular weight PBDEs were formed (three pentaBDE congeners were identified, but almost no tetraBDEs were found). In addition to direct photolysis, aerosol-borne 2,2',4,4',5,5'-hexaBDE was found to react with hydroxyl radicals, and a preliminary value for the rate constant for the reaction was determined as 2x10-12 cm3 molecule-1 s-1 at 7ºC. The products from the reaction with hydroxyl radicals were not determined.

would not be expected to receive full sunlight exposure for the majority of the day and that windows filter out a substantial fraction of light in the UV-A region. They estimated a more realistic half-life of around 200 days based on two hours' exposure to sunlight per day.

In the experiments with spiked dust, increasing concentrations of several hepta-, octa- and nonaBDEs were evident as the concentration of decaBDE decreased with exposure. The degradation products formed included all three nonaBDEs (BDE-206, BDE-207 and BDE-208), at least six octaBDEs (BDE-196, BDE-197, BDE-200/203, BDE-201, BDE-202[6] and one unknown congener) and three heptaBDEs (BDE-183 and two unknown congeners). BDE-202 has also been detected in anaerobic degradation studies (e.g. Gerecke et al., 2005 (see Section 3.1.2.3)) and might be a possible marker for transformation of decaBDE as it is not a known component of any commercial PBDE product.

In the experiment with the standard reference material there was some evidence for increasing concentrations of BDE-208, BDE-201 and BDE-202 during the test but the results are more difficult to interpret owing to the larger number of congeners initially present in the starting material.

The ratio of BDE-197 to BDE-201 in the samples was also investigated. The relative proportion of these two octaBDE congeners has been found to be relatively constant in commercial octaBDE products (ranging from 28 to 35 in two products). The ratio of these two congeners in the spiked dust samples was found to be 0.9 after 20 hours' exposure to light and 0.6 by the end of the exposure period (the average ratio over the entire exposure period was 0.8). In the standard reference material used in the study the ratio of these two congeners decreased from a value of 4.8 to a value of 1.2 over the 200-hour exposure period, with the trend in the ratio appearing to be towards that seen in the spiked dust samples with increasing exposure. This trend was thought to result from either a faster formation of BDE-201 relative to BDE-197 or the slower degradation of BDE-197 relative to BDE-201.

A mass balance analysis was also performed. At the start of the study the total amount of PBDEs present in the standard reference material was 2,300 pmol (primarily as decaBDE). At the end of the exposure period approximately 850 pmol (or 38%) of the decaBDE mass had been lost or degraded. Of this amount, 300 pmol was accounted for by the formation of lower PBDE congeners (around 240 pmol (~28% of the mass loss) as nonaBDEs, around 56 pmol (~6.5% of the mass loss) as octaBDEs and around 4 pmol (~0.5% of the mass loss) as heptaBDEs). The fate of the remaining 550 pmol (i.e. 65% of the mass that was lost) is currently unknown (possibilities include loss by volatilisation of decaBDE or degradation products, adsorption of decaBDE or degradation products to the walls of the test vessels, non-extraction of decaBDE or degradation products from the dust samples or formation of unknown degradation products). The mass balance also showed that there was an overall reduction in the total amount of PBDEs present in the dust samples. For example in the spiked dust the total concentration of all PBDEs detected was 2,310 pmol/g dry weight and this had fallen to 1,750 pmol/g dry weight (around 76% of the initial amount present) after 200 hours (a similar reduction in the total amount of PBDEs was also evident in the experiments with the standard reference material).

[6] No information on the source, synthesis or confirmation of the identity of BDE-202 was given in the paper. BSEF (2009) queried whether the identification of this specific congener was certain, and indicated that no certified analytical standard for BDE-202 was available at the time of the study. Most studies have used samples of BDE-202 (and BDE-196 and BDE-197) that were gifts from a university or other researchers. Lack of information on this material may introduce some uncertainty to the identification and quantification of this congener. However, the synthesis and characterisation of BDE-202 appears to be given in a paper by Teclechiel et al. (2007) and so this may in practice be less of a concern than suggested by BSEF (2009).

This study was well-conducted, and demonstrates that exposure of decaBDE on small particulates to sunlight can result in the formation of heptaBDEs (accounting for 0.5% of the decaBDE mass lost over 200 hours), as well as nona- and octaBDEs and other (unknown) transformation products. On a molar basis, the percentage formation over 200 sunlight hours (equivalent to 25 or 100 days assuming eight or two hours' sunlight exposure per day, respectively) was 10% for nonaBDEs, 2.4% for octaBDEs and around 0.2% for heptaBDEs. The sample cleaning and spiking method could have some influence on the availability of the substance for reaction; decaBDE in natural dusts is likely to be more intimately associated with the particle matrix. On the other hand, transformation might have been more extensive had the particles been exposed in a suspended state.

c) Stapleton (2006) investigated the photodegradation of decaBDE on household dust by natural sunlight in a separate experiment to the one summarised above. The dust used was again a standard reference material that had been pre-cleaned to remove all native PBDEs before spiking with decaBDE as a solution in toluene following the methodology outlined above. The exposure of the spiked dust was carried out in an identical way as in the above study, except that the exposure was carried out during the hours of 9 a.m. to 4 p.m. for up to a total of 90 hours. Control samples (wrapped in aluminium foil) were kept in the laboratory. The experiment was carried out between 29 July and 24 August 2004 and the temperature during the exposure ranged between 20.3 and 30.8°C (mean 27.4°C). The mean incoming solar irradiance was determined to be 597 W/m^2 during the study. At the start of the study, the initial concentration of decaBDE in the dust was 2,180 µg/kg dry weight. After 90 hours' sunlight exposure the concentration had decreased to 1,570 µg/kg dry weight, indicating that around 28% of the test substance had degraded. The corresponding concentration in the dark control at the end of the exposure period was 1,990 µg/kg dry weight which was not statistically significantly different ($p=0.05$) from the starting concentration. Assuming a first order degradation process, the half-life for removal was estimated to be around 216 sunlight hours (assuming 8 hours' sunlight exposure per day, this is equivalent to a half-life of approximately 27 days).

   The concentrations of all three possible nonaBDE congeners were found to increase with increasing exposure time, along with six octaBDE congeners (three of these were identified as BDE-196, BDE-197 and BDE-203) and an unidentified heptaBDE congener. The concentrations of these transformation products were still increasing at the end of the 90-hour exposure period. Mass balance calculations indicated that around 17% of the starting mass of decaBDE was not accounted for by these products and it was hypothesised that this loss of mass from the system could have been due to either volatilisation of transformation products or formation of as yet unidentified degradation products. The results of this experiment support those reported by Stapleton and Dodder (2006 [ABST] and 2008), with similar drawbacks.

d) The photolysis of decaBDE in ground plastic samples has been studied by Kajiwara et al. (2007 [ABST] and 2008). (This study was not summarised in previous EU risk assessment reports.) The experiments were carried out using samples of high impact polystyrene (HIPS) (to which decaBDE was added) and composite samples of used television cases (which already contained decaBDE):

   - The HIPS samples were prepared by adding 50 g of HIPS to 500 ml of toluene containing 100 µg/ml of decaBDE. The mixture was shaken overnight to facilitate complete dissolution of the HIPS. The solvent was then evaporated in the dark and the HIPS sample pulverized and screened to produce a fine powder (particle diameter between 106 and 300 µm) for use in the experiments. The specific surface areas of the sample was 0.222 m^2/g.

   - Fifty television cases were obtained from a recycling plant in Japan. They were crushed to produce particles with a diameter below 2 mm and homogenised in a large volume mixer. A sub-sample was further pulverized and screened to give a

fine powder (again with a diameter between 106 and 300 µm). The specific surface area of the sample was 0.275 m^2/g.

The photolysis experiments were carried out using 0.30 g of the powdered plastics. The powder was placed in quartz tubes, sealed and exposed to natural sunlight for up to 224 days (from September 2006 to May 2007 in Tsukuba, Japan (36°02'N, 140°07'E)). During the exposure the tubes were kept in a temperature-controlled glass room at 22°C and the tubes were constantly rotated (twelve revolutions per minute). Dark control experiments were also carried out. In addition, some samples of the HIPS containing decaBDE were hydrated with water (0.5 ml per tube) before the start of the experiment to investigate the effect of moisture on the photodegradation.

At various times during the exposure, one dark control and duplicate exposure samples were analysed for the presence of PBDEs (a total of twenty-five congeners were analysed covering di- to decaBDE), polybrominated dibenzo-*p*-dioxins (five congeners were analysed covering tri-, tetra-, penta-, hexa- and octa- congeners) and polybrominated dibenzofurans (seven congeners were analysed covering di- to octa-congeners). Positive identification was made by comparison with authentic standards. In addition, several unknown peaks were also found to be present and these were assigned to the appropriate homologue group.

In the experiments with HIPS to which decaBDE was added, the initial concentration of decaBDE was 1,300 mg/kg (i.e. 0.13% by weight), with smaller amounts of nonaBDEs (140 mg/kg), octaBDEs (4.4 mg/kg) and heptaBDEs (0.93 mg/kg) and no detectable di- to hexaBDEs. DecaBDE was found to disappear from the HIPS sample on exposure to sunlight, with around 50% loss after around seven days. No loss of decaBDE was evident in the dark controls throughout the experiment and so the loss seen from the HIPS samples exposed to light was presumed to represent photodegradation. The results are summarised in Figure 1.

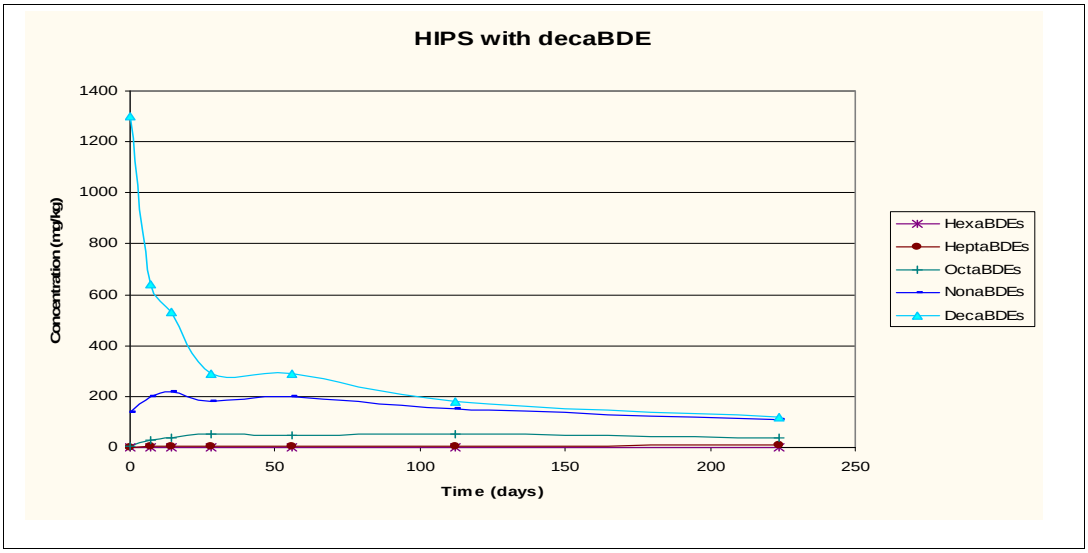


Figure 1: Formation of PBDEs during photolysis of decaBDE adsorbed to HIPS (after Kajiwara et al., 2008)

The concentration of several lower PBDE congeners (hexa- to nonaBDEs) increased after one week of exposure, indicating that they were products of the photodegradation of decaBDE. However, from one week onwards the concentrations of these congeners remained relatively constant (or decreased slightly) while the concentration of decaBDE continuously declined. By the end of the study the concentration of total PBDEs found in the sample was less than 20% of the initial concentration and the proportion of the total PBDEs that was attributable to decaBDE had changed from around 90% at the start of the study to around 44% by the end. No di- to pentaBDE congeners were seen at any time point.

Assuming a first order decay, the half-life of decaBDE under these conditions was estimated to be 51 days. The degradation rate in the experiments with added water was reported to be faster than seen in HIPS alone although data were available after 112 days' exposure only and few other details of the experiments with water were given.

No polybrominated dibenzo-*p*-dioxins were detected during the experiment with HIPS and decaBDE. However, the concentration of total polybrominated dibenzofurans was found to show a marked increase (greater than 40 times) over the first seven days of the study (see Figure 2). At the start of the study, only traces of octabromodibenzofuran (0.15 mg/kg) and heptabromodibenzofuran (0.099 mg/kg) were determined in the samples but after one weeks' irradiation tri- to hexabromodibenzofurans were also found to be present at concentrations ranging from 0.036 to 3.1 mg/kg. The concentrations of the brominated dibenzofurans then decreased with increasing irradiation, indicating that these substances were themselves subject to photodegradation.

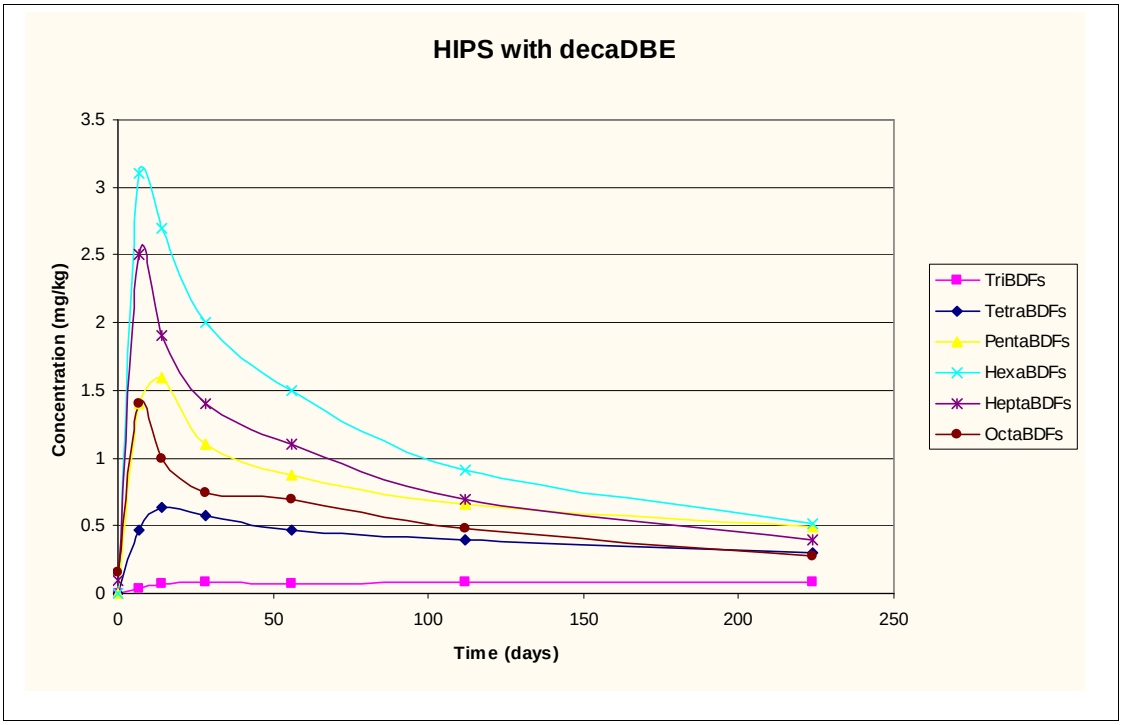


Figure 2: Formation of dibenzofurans during photolysis of decaBDE adsorbed to HIPS (after Kajiwara et al., 2008)

In contrast, the experiments with the television casing samples showed no clear degradation of decaBDE. The initial concentration of decaBDE in the samples was 96,000 mg/kg (i.e. 9.6%) and the concentrations measured between day seven and day 224 were in the range 96,000 to 110,000 mg/kg. Although no significant loss of decaBDE was seen in these studies, the concentrations of di- to octabromodibenzofurans were found to show a continuous increase over the course of the study, with the level of total brominated dibenzofurans increasing approximately twenty times over the initial level present by the end of the study. The study authors postulated that this difference in behaviour might have been due to the presence of other additives in the television casings, including colouring agents, UV absorbers and stabilizers. These could have minimised radical formation and affected the amount of light to which the decaBDE present was exposed (the casing samples were black but the HIPS samples were creamy white in colour). In addition, the high concentration of decaBDE present in these samples made it difficult to detect any small amount of degradation of decaBDE that may have occurred. The increase in the levels of brominated dibenzofurans during the experiment was thought to result from the photodegradation of decaBDE.

Mass balance calculations showed that in the experiments with decaBDE in HIPS, over 90% of the decaBDE initially present was degraded in 224 days. However the identified products (lower PBDEs and brominated dibenzofurans) only accounted for around 1.1% and 0.24% respectively of the initial decaBDE at the end of the study. This implies that other, as yet unidentified products, could be major photodegradation products of decaBDE.

This is considered to be a reliable study. Although it was carried out using ground plastic samples, it is relevant to this dossier because dust containing decaBDE could be generated from plastic articles in use or at disposal. The study shows that a range of phototransformation products can be formed when sunlight falls on decaBDE adsorbed to plastic particles with a large surface area, including hepta- and hexaBDEs. However, the amounts formed appear to be very small, with PBDEs and brominated dibenzofurans only accounting for around 1.1% and 0.24% respectively of the decaBDE initially present after 224 days. Over shorter timescales of a few days, there was a clear increase in the concentration of nonaBDEs (by about a factor of three) as well as a smaller increase in octaBDEs. Other, as yet unidentified products, could be major photodegradation products of decaBDE. It is possible that transformation might have been more extensive if the particles had been suspended in air.

e) Kajiwara & Takigami (2010) [ABST] investigated the photolytic behaviour of a commercial decaBDE product using a sample of treated curtain textile. (This study was not summarised in previous EU risk assessment reports.) The sample (100% polyester) contained decaBDE at a concentration of 120 g/kg. The experiment was performed from November 2007 to November 2008 at Tsukuba, Japan (36°02'N, 140°07'E). The textile sample, 120 cm long and 20 cm wide, was hung over a window in a temperature-controlled glass room (22°C) to achieve uniform sunlight exposure. A strip of material (approximately 5 centimetres long) was cut from the bottom edge every four weeks, at approximately the same time of day. The sub-samples were wrapped in aluminium foil, and stored at room temperature in the laboratory until chemical analysis for PBDEs, polybrominated dibenzo-*p*-dioxins (PBDDs) and furans (PBDFs) by high resolution GC/MS. Although there was no clear disappearance of decaBDE or formation of lower molecular weight PBDEs during the 371-day exposure, the concentrations of PBDF congeners showed a continuous increase during the experimental period. Total PBDF concentrations in the curtain sample after the one-year exposure reached 17 µg/g, which was approximately seven times the initial level (2.4 µg/g). Of the congeners analyzed, octaBDF was the predominant congener throughout the exposure, comprising 50% to 80% of the total PBDFs.

   Although there was a lack of replication and no controls, this study suggests that treated curtains may be a source of PBDF contamination in indoor air and dust, through the photodegradation of decaBDE.

f) Raff and Hites (2007a [ABST] and 2007b) carried out a modelling study to investigate the relative importance of various atmospheric removal processes for PBDEs by comparing atmospheric residence times expected for reaction with hydroxyl radicals with direct photolysis. (This study was not summarised in previous EU risk assessment reports.) The study suggested that direct photolysis is likely to be the dominant loss process for PBDEs in the atmosphere. However, it was noted that this is only likely to be true for molecules present in the gas phase as a number of physical and chemical effects may hinder the direct photolysis of PBDEs adsorbed to particles. When the effect of partitioning to atmospheric particulates was taken into account (assuming that PBDEs in the particulate phase do not undergo photolysis) the analysis suggested that the congeners present mainly in the gas phase will be removed from the atmosphere primarily by direct photolysis but those that are bound mainly to atmospheric particulates, as is the case with decaBDE, will not be significantly degraded by sunlight. Rather, they are likely to be removed from the atmosphere mainly by wet and dry deposition.

Evidence for this hypothesis was sought by comparison of the PBDE congener profiles found in surface sediment from Siskiwit Lake located on Isle Royale in Lake Superior with the congener pattern in air (particulates plus vapour phase) from Eagle Harbour located near the shore of Lake Superior. Lake Siskiwit is a remote lake with few visitors that receives no water from Lake Superior and hence atmospheric deposition is the main source of PBDEs in the lake. The most abundant congener present in the lake sediment was decaBDE, accounting for 95% of the total PBDEs. There was a higher proportion of decaBDE in the sediment compared to that in the air, suggesting some depletion of the lower PBDE congeners by photolysis (and/or reaction with hydroxyl radicals) compared to decaBDE in the air sample before deposition. (If atmospheric deposition was the only loss process for PBDEs from air then the congener profile in the sediment would be expected to be similar to that in air.)

g) Thomas and Jones (2007) determined the levels of decaBDE and nonaBDEs (BDE-206, BDE-207 and BDE-208) in a total of nine two-day air samples collected at a single site over a period of two months during May to June 2007. (This study was not summarised in previous EU risk assessment reports.) The sampling site was a well characterised field and meteorological station at Hazelrigg in north-west England. The site was situated in a semi-rural area (predominantly grassland) at 54°2' N, 2°46' W and at a height of 94.1 m above sea level. A city (population of 50,000) lies approximately 4 km from the site, and the site is 10 km from the coast. The samples were collected by drawing air through a glass-fibre filter (to collect particulate matter) followed by a polyurethane foam filter (to collect vapour phase chemical). The reported results represented the total (i.e. particulate plus vapour phase) concentration, and a comparison was made with the findings of a similar study from the same location in 2005 (Thomas and Jones, 2006 – this study was summarised in ECB, 2007a). The quality control/quality assurance procedures included the routine analysis of field blank samples and exposure to UV-light was minimised during the extraction and clean-up procedure.

   DecaBDE was found to be present in all samples in the range 6.8 to 89 pg/m^3. The geometric mean concentration was 18 pg/m^3, which was not statistically significantly different from that found in 2005 (15 pg/m^3). The geometric mean concentration of nonaBDEs was an order of magnitude lower, and there was also no statistically significantly difference between 2007 and 2005. In contrast, a statistically significant decrease (95% confidence limit) in the concentration of two octaBDE congeners (BDE-196 and BDE-197) had occurred in 2007 compared with 2005 (geometric mean concentrations were 0.15 and 0.21 pg/m^3 for the two congeners in 2007, compared to 0.29 and 0.47 pg/m^3, respectively, in 2005). This result is interesting because it shows that the levels of these two octaBDE congeners in air are falling (presumably as a result of restrictions in the EU on the use of the commercial octaBDE products) while the levels of decaBDE and nonaBDE have remained relatively constant over the same time period. This could suggest that photolysis or atmospheric degradation of decaBDE does not make a significant contribution to the current levels of these two octaBDE congeners in these air samples. At the same time, this study cannot be used as evidence that this mechanism is not relevant (since other congeners might be formed, and the current levels might mask a low level of formation).

h) Wilford et al. (2008) investigated the levels of decaBDE and nonaBDEs (BDE-206, BDE-207 and BDE-208) in air particulates sampled at the same semi-rural site in north-west England as used by Thomas and Jones (2007). Samples were collected between 17 April and 20 May 2004. (This study was not summarised in previous EU risk assessment reports.) The median total nonaBDE concentration was around 45% of the concentration of decaBDE. The nonaBDEs were therefore present in much higher amounts than would be expected based on their reported occurrence in the commercial decaBDE products (3%).

The relationship of the concentration of nonaBDEs with the concentration of particles within selected size bands was analysed. There were strong indications that nonaBDEs were associated mainly with the larger size particles (>3 μm diameter) which are associated with windblown dusts from mechanical processes rather than smaller particles formed from soot and condensed vapours. This suggested that the nonaBDEs and decaBDE were predominantly attached to particles formed by abrasion of articles in use rather than volatilisation from articles.

The study also determined the levels of BDE-183 (a major congener in the commercial octaBDE product). A significant correlation ($p<0.01$) was found between decaBDE, BDE-208, BDE-207 and BDE-206 but not with BDE-183, suggesting that the nonaBDEs were derived from the same source as decaBDE.

This analysis leads to two possible explanations for the observed congener pattern: debromination of decaBDE to nonaBDEs or ongoing emissions from the past use of commercial decaBDE products with higher nonaBDE contents. It is known that the purity of the products supplied by the three principal European importers has been above 97% since before 1999 (BSEF, 2009). Photodegradation might therefore be the more plausible explanation for this observation.

i) Gearhart and Posselt (2006) carried out a survey of the levels of PBDEs in samples of windscreen films and dust from car interiors in the United States. (This study was not summarised in previous EU risk assessment reports.) Samples were collected by wipe sampling from the front windscreen of 111 vehicles. Thirteen composite samples were analyzed, with each composite sample consisting of between 6 and 10 samples from individual cars from the same manufacturer. Dust samples were collected from the carpets and seats of around 21 vehicles using a vacuum cleaner. Two composite dust samples were analyzed.

   DecaBDE was found to be the dominant PBDE congener in the dust samples, but was only a minor component in the windscreen films (accounting for around 1.6% of the total PBDEs present). The low concentrations on the windscreen films could possibly be explained by a lower volatility of decaBDE from the treated furnishings than other congeners, or photolytic degradation of the substance on the windscreen. The authors pointed out that studies looking at PBDE levels in window films from various buildings had generally shown a higher contribution from decaBDE (typically 50-80% of the total PBDEs present). The study findings were consistent with increased photolytic degradation due to sunlight exposure (possibly combined with higher temperatures) on car windscreens. This study provides circumstantial evidence of debromination, and the transformation products are not known. However, it provides an example of a scenario where the reaction might be important.

j) Harrad and Abdallah (2011) measured PBDEs in a preliminary study of dust from passenger cabins and boots of fourteen cars in the UK. Possible phototransformation of decaBDE was indicated by significantly higher ($p<0.05$) concentrations of BDE-202 (an octaBDE) in cabin dust. In contrast, Lagalante et al. (2011) found no significant photochemical degradation of decaBDE in a laboratory study of dust sampled from inside sixty-six vehicles in the United States after 56 days of constant UV-A irradiation (decaBDE was the dominant PBDE congener in the samples, with a median level of 8.12 μg/g). (These studies were not summarised in previous EU risk assessment reports.)

k) Meyer et al. (2012) collected snow cores from the Devon Ice Cap in Nunavut, Canada. The cores correlated with the period from approximately 1993 to 2008. Samples were extracted under clean room conditions, and analyzed using gas chromatography-negative ion mass spectrometry for twenty-six PBDEs. DecaBDE was the major congener present in all samples followed by the three nonaBDEs (89% and 7% of the total, respectively). DecaBDE concentrations were in most cases significantly correlated ($p < 0.05$) to tri- to nonaBDE homologues, and the strength of the correlations increased with increasing degree of bromination. The relatively

high contribution of nonaBDEs (differing from the composition of typical commercial products), the BDE-197/BDE-201 ratio plus the detection of two octaBDEs (BDE-201 and BDE-202) were thought to provide evidence of debromination prior to or after deposition.

Discussion

DecaBDE is likely to be mainly adsorbed to particulates in the atmosphere, and so data relating to phototransformation on particulates are the most relevant.

The studies discussed above present somewhat conflicting information about the relevance of atmospheric phototransformation for decaBDE in terms of the potential to form lower PBDEs of concern. Interpretation of the studies is complicated by the range of light intensities and wavelengths that were used. The most relevant studies are likely to be those that used exposure to natural sunlight. On the one hand, experiments demonstrate that small amounts of hepta- or even hexaBDEs may be formed when decaBDE adsorbed to particulates is exposed to sunlight over sufficient timescales. Stapleton and Dodder (2008) (supported by Stapleton, 2006) found that the percentage formation over 200 sunlight hours (equivalent to 25 or 100 days assuming eight or two hours' sunlight exposure per day, respectively) was 10% for nonaBDEs, 2.4% for octaBDEs and around 0.2% for heptaBDEs (on a molar basis). Kajiwara et al. (2008) also found that nonaBDEs (and smaller amounts of octaBDEs) could be formed over timescales of a few days to a week when decaBDE adsorbed to plastic particles was exposed to sunlight. Over longer exposure periods, there is evidence of further degradation of the lower molecular weight congeners (Kajiwara et al., 2008). Other as yet unidentified products could be major photodegradation products of decaBDE, and Kajiwara et al. (2008) found that tri- to octabromodibenzofurans can also be formed in small amounts. The study of Palm et al. (2003) appears to have been of too short a duration to detect this level of degradation.

In contrast, monitoring studies (Raff and Hites (2007a [ABST] and 2007b), Wilford et al. (2008) and Thomas and Jones (2007)) do not provide evidence for the formation of lower molecular weight PBDE congeners in air, other than possibly nonaBDEs. The interpretation of such studies is difficult because current PBDE levels might mask a low level of transformation. However, these findings could also be related to the length and intensity of exposure of decaBDE to light whilst in the air, and mitigating factors that might hinder direct photolysis such as shielding by particulates, photophysical quenching by neighbouring molecules in the condensed phase and enhanced recombination in a 'solvent cage' within the aerosol. The long-term fate of the transformation products might also be important since there is some evidence that they may be also susceptible to photodegradation and/or reaction with hydroxyl radicals (e.g. Kajiwara et al., 2008).

Overall, the atmospheric residence time of decaBDE is expected to govern the relevance of this pathway in practice. This is discussed in Section 3.2, and whilst larger particles may be removed in minutes, finer particles (with a diameter around a few micrometres) might remain airborne for hours or days, provided that they are not removed by wet deposition. The experimental evidence suggests that phototransformation to at least nonaBDEs in amounts of several per cent w/w could occur over such timescales, and this is supported by the findings of Wilford et al. (2008) and Meyer et al. (2012). These will ultimately be deposited to sediments and soils. Smaller amounts of other substances such as octa- and heptaBDEs and brominated dibenzofurans might also be formed in some circumstances, although it is not possible to assess the likely extent of this.

Phototransformation could be important in certain exposure scenarios, for example in car interiors (Gearhart and Posselt, 2006) and curtains (Kajiwara & Takigami, 2010 [ABST]), although information is limited. It might also be relevant for particles that are deposited to terrestrial or aquatic surfaces, and this is considered in the following sections.

3.1.1.2.2 Phototransformation in water

The registration dossiers do not provide any information on this end point.

A large number of studies have been performed that demonstrate phototransformation of decaBDE when dissolved in various organic solvents or water (for example Norris et al. (1973 and 1974), Watanabe et al. (1986), Watanabe and Tatsukawa (1987), Ohta et al. (2001) [ABST], Olsman et al. (2002 [ABST] and 2006), Eriksson et al. (2001a [ABST] and 2004), da Rosa et al. (2003), Palm et al. (2003), Peterman et al. (2003) [ABST], Bezares-Cruz et al. (2004), Kalbin et al. (2005), Hagberg et al. (2006), Geller et al. (2006) [ABST], Kuivikko et al. (2006 [ABST] and 2007), Mas et al. (2008a), Zeng et al. (2008 & 2010), Christiansson et al. (2009), Sun et al. (2009), Xie et al. (2009) and Shih and Wang (2009)). Studies involving organic solvents show that decaBDE can readily lose bromine atoms, with the reaction rate decreasing as the number of bromine atoms declines. However, in almost all of these cases, the results cannot be extrapolated directly to the environment. Firstly, the organic solvent itself could act as a hydrogen donor to any radical species formed from the initial cleavage of the carbon-bromine bond, and so the products formed, and rate of reaction, seen in such studies may be different to those involving water. Secondly, the water solubility of decaBDE is very low (below 1 µg/l), and it is likely to be associated mainly with particulate phases. Photolysis reactions on solid surfaces are therefore more relevant to the actual fate of decaBDE in the environment.

This section therefore only summarises studies that used water, solids suspended in water, or sediment.

a) Norris et al. (1973 and 1974) exposed a test substance consisting of 98% decaBDE and 2% nonaBDE in water to natural sunlight. Desiccators, each containing 10 g of decaBDE and 8 litres of water, were fitted with a polyethylene film lid and placed side by side on the roof of a building. Over the 98-day exposure period, the total bromine content in water of the exposed sample was found to increase from 2.6 mg/l at 31 days, to 5.6 mg/l at 66 days and then 7.3 mg/l at 98 days. The level of bromine in the water from the unexposed control was 0.2 mg/l after 98 days. GC analysis of xylene extracts showed several new unidentified peaks compared to controls after 98 days' exposure. These were more volatile (i.e. had shorter GC retention times) than the 4-bromodiphenyl ether standard and so were not due to other PBDEs. Given that only a small amount of the test substance would have been dissolved, only a small fraction of the total decaBDE present is likely to have been exposed to sunlight under the conditions used (i.e. the substance in solution and/or the surface layer of the solid). This study is therefore not relevant.

b) Örn (1997) reported that exposure of decaBDE dispersed as a thin layer on sand to sunlight (midsummer 1990) resulted in the formation of debrominated products. In another experiment when water was added to the sand, brominated phenols were observed as well as lower PBDEs. Few details are available, so the reliability of this study cannot be assessed. It is possible that is was part of the series of experiments by Sellström et al. (1998) [ABST] reported below.

c) Sellström et al. (1998) [ABST] and Tysklind et al. (2001) [ABST] both report a study of the photolytic degradation of decaBDE using a variety of media, including sediment from Lake Vanem, Sweden, with low contaminant levels (no further details of sediment characteristics are provided). The composition of the test substance was not reported, but it contained nonaBDEs and traces of octaBDEs. A solution of decaBDE in toluene was added to dried sediment, and the solvent was allowed to evaporate in the dark. The samples were reconstituted with water and then sub-samples of sediment were placed in pyrex tubes and irradiated for up to 32 hours (using four mercury UV-lamps fitted with filters to give a spectrum as close as possible to natural sunlight, at an irradiance intensity of 1.6 mW/cm^2) or 96 hours (using natural sunlight, at an irradiation intensity at mid-day 2.3 mW/cm^2). The irradiance from 24 hours sunlight corresponded to that of around 9 hours of artificial

light. Experiments were performed in triplicate and each series consisted of blanks, dark controls and the samples. The analysis of degradation products formed was carried out by gas chromatography-mass spectrometry using negative chemical ionisation and monitoring for the bromine ions formed (m/z -79 and -81). Sample extraction and preparation was carried out in the dark.

Reductive debromination was observed, with an increase in the amounts of nona-, octa- and heptaBDEs compared to controls, along with lower PBDEs (it was not always possible to identify the exact congeners formed due to the lack of suitable reference standards). The half-life for removal of decaBDE in sediment was estimated to be 100 hours (Tysklind et al., 2001 [ABST]). Söderström (2003) and Söderström et al. (2004) provide a more detailed discussion of the products formed in this study, and show that 2,2'4,4',5,6'-hexaBDE and possibly 2,2',4,4',5,5'-hexaBDE were detected in sediment. Söderström (2003) also estimated a sediment half-life of 53 hours. The mass balance, based on the amounts of lower molecular weight PBDEs, was low indicating that compounds other than PBDEs were being formed as well.

This reliable study provides good evidence for the formation of hepta- and hexaBDE congeners in freshly spiked sediment following exposure to light over 96 hours under laboratory conditions.

d) Jafvert and Hua (2001a) (also reported in Hua et al., 2003) investigated the degradation of decaBDE on hydrated surfaces (quartz glass and silica particles (sand)), humic acid-coated silica particles and also adsorbed to glass surfaces in contact with aqueous solutions. Some indirect aqueous photolysis studies were also carried out using humic acid as a source of photolytically produced free radicals in solution. Two different light sources were used: 3,000 Å lamps (giving light in the 280-320 nm (UV-A and B) wavelength range); and natural sunlight (from the end of March to the beginning of May 2001 at Purdue University, West Lafayette, Indiana, USA (40° 26' N, 86° 55' W), between 9 a.m. and 5 p.m. on clear or partly cloudy days).

Various analytical methods were used to determine the extent of degradation seen in the experiments and identify any PBDE products that were formed. The main method was High Performance Liquid Chromatography (HPLC) with UV detection. This was used to determine the parent decaBDE concentration during the course of the experiments, and as a screen to determine if any other PBDEs were possibly present. The other PBDEs were tentatively identified by comparison of peak retention times with those of hexa- to nonaBDE congeners present in some commercial PBDE products. However, as the HPLC method provides no structural information, and UV detection is relatively non-specific, the actual identity of the products formed could not unambiguously be determined by this method. A more detailed GC/MS analytical method was used in some exposures to positively identify any lower molecular weight PBDEs formed and, where available, these results are included in the discussion below. In addition, the amount of free bromide ion produced during the photolysis was determined at various times during the exposures.

For each experiment, a bromine mass balance was determined at various time points based on the measured concentrations of parent substance and bromide ion using the following equation:

$$\% \text{ Bromine recovery} = \left(\frac{10 \times [\text{decaBDE}]_t + [\text{Br}^-]_t}{10 \times [\text{decaBDE}]_0} \right) \times 100$$

where: $[\text{decaBDE}]_t$ = concentration of decaBDE at exposure time *t*.

$[\text{Br}^-]_t$ = bromide ion concentration at exposure time *t*.

$[decaBDE]_0$ = initial concentration of decaBDE.

This allowed the amount of bromine present (expressed as a percentage of the initial amount of bromine added as decaBDE) in products that was not either decaBDE or bromide ion to be determined during the exposures. The actual identity of these products is not known, but would include other PBDEs.

Six series of photolysis experiments were performed, and these are summarised below.

- The first part of the study determined the absorption spectra of several PBDEs in ethanol, including decaBDE (98% purity). This solvent was chosen for solubility reasons, but it was thought that the important features of the spectra would be the same in water. The di- and tetraBDEs were found to absorb minimal light at wavelengths above 300 nm, whereas both decaBDE and a commercial octaBDE product absorbed light up to around 325 nm. At 280 nm, all the compounds investigated showed similar molar absorptivities (molar absorption coefficients (ε) were all in the range 2,000 to 3,000 M^{-1} cm^{-1}). The absorption spectrum for decaBDE indicated that it may be susceptible to photodegradation with light at environmentally relevant wavelengths.

- The first series of photolysis experiments involved the solar irradiation of decaBDE adsorbed to sand (silica). The spiked sand was prepared by adding a total of 50 ml of a solution of decaBDE in toluene (concentration 2.0×10^{-3} M) to 500 g of sand in 1 ml increments. After each 1 ml addition, the sand was mixed with a steel spatula under a stream of nitrogen gas (to volatilise the solvent). Once all the toluene solution had been added in this way the spiked sand was placed in a vacuum desiccator for 24 hours. This gave a decaBDE concentration of 3.8 g/kg sand. The experiments were carried out using twenty petri dishes each containing 7.015 g of the spiked sand and 9 ml of water. The water was replenished by weight at the end of each day. Six control sand samples were prepared in a similar way but without addition of decaBDE. The six control samples and ten of the spiked samples were then left uncovered and exposed to natural light daily between 10 a.m. and 4 p.m. The remaining ten spiked samples were placed in a dark container to act as the dark controls. At various times during the experiments two dark controls, two blank controls (without decaBDE added) and two exposed samples were sacrificed for analysis for the presence of decaBDE.

  It was found that the amount of decaBDE present in the sand declined by around 10% during the first 12 hours of irradiation, with a slower disappearance occurring over longer exposures. After 84 hours' irradiation approximately 20% of the initial decaBDE had disappeared from the sand. However, a similar disappearance was seen from the dark controls (the concentrations in the exposed samples and the dark control samples were statistically similar at all time points over the 84-hour exposure period). Therefore it was concluded that no or insignificant photodegradation of the substance occurred over 84 hours in this experiment, although it appears that no bromide analysis was carried out. As this experiment was carried out with sand particles, only the decaBDE on the top few millimetres of the sand would be expected to be exposed to light. Given the disappearance seen in the dark control samples, the significance of these findings (and the loss mechanism itself) is unclear.

- The second series of experiments looked at the solar irradiation of decaBDE adsorbed to humic acid-coated sand. The sand (-50 to +70 mesh) was coated with a commercial humic acid at a concentration of 2.57×10^{-3} g humic acid/g sand. The humic acid-coated sand was then prepared in a similar fashion to the sand samples in the previous exepriment (in this case 4.8 ml of a 1.0×10^{-3} M solution of decaBDE in toluene was added to 30 g of sand in 0.5 ml increments) giving a final concentration of 0.15 g/kg sand (1.6×10^{-4} mol/kg sand). The organic carbon content of the sand was 0.07%. The exposures were carried out

using sealed quartz cuvettes containing 0.5 g of the spiked humic acid-coated sand and 2 ml of water. The depth of sand was around 3 mm. The samples were exposed to sunlight from 9 a.m. to 5 p.m. daily. Blank controls and dark controls were also run as before.

After 96 hours' exposure to sunlight over 16 days, around 12% of the decaBDE had disappeared from the sand, showing that humic acid attenuated decay. The dark controls were found to exhibit slight fluctuations in decaBDE concentration but generally showed no loss. Some indications of the presence of transformation products were found by HPLC analysis of the exposed samples (and bromide ion was found to accumulate during the exposure) but the identity of these peaks was unconfirmed (they were thought to be nona- and octaBDE congeners). The bromine mass balance at all time points indicated that over 95% of the bromine was present as either decaBDE or bromide ion, showing that the amounts of other organobromine compounds present would be small (below 5% of the bromine present) under these conditions. However, it was noted that the HPLC peak area had increased compared to the experiment without humic acid. Again, sunlight penetration was limited. The results can be explained by the attenuation of relevant wavelengths by humic acid, and the fact that humic acid is a hydrogen donor, promoting reduction rather than condensation polymerisation reactions.

- A more detailed GC/MS analysis was carried out by Jafvert and Hua (2001b) using two replicate exposures under the same conditions as indicated above (but with a test duration of 72 hours). The concentrations of 43 individual PBDE congeners, as well as the total di- to octaBDE congeners, were determined. The results are presented in detail in EC (2002). The authors stated that the results were inconclusive as to whether octa- and nonaBDEs were formed, but did not comment on the formation of lower molecular weight PBDEs. A simple analysis was carried out for the purposes of the ESR assessment by comparing the mean and standard deviation of the concentrations of each PBDE congener at the start and end of the experiment. There was considerable variation within the concentrations found in the two replicates, which made it difficult to draw definite conclusions. However, there was evidence that some PBDE congeners, in particular 2,2'4,4',6,6'-hexaBDE (BDE-155), were formed in the experiment (i.e. the mean concentration minus the standard deviation at 72 hours was higher than the mean concentration plus standard deviation at the start). For some congeners (for example several other hexa- and heptaBDEs) the mean concentration at 72 hours was higher than at the start but there was overlap of the standard deviation ranges. The very small sample size and high variability in the results means that the findings are uncertain, but they provide an indication that several hexa- and heptaBDE congeners might have been formed over 72 hours, at very low concentrations/yields (for example, around 1,000 ng/kg sand for BDE-155).

- The third series of experiments investigated the solar irradiation of decaBDE adsorbed to quartz tubes containing humic acid solution. The samples were prepared by adding 1 ml of a 2×10^{-5} M solution of decaBDE in toluene in a cylindrical quartz tube and then evaporating off the solvent under a stream of nitrogen gas. As the solvent was evaporated, the tubes were rotated at an angle of 45° to ensure even coverage of the surface. The amount of decaBDE present in the tube was 0.019 mg. A solution of humic acid in water (2 ml of a 100 mg/l solution) was then added to each tube. Exposure was carried out using natural sunlight (9 a.m. to 5 p.m.). Dark controls and blank control samples were also prepared.

  The results from the experiments showed a decrease in the decaBDE concentration and increase in bromide ion concentration with irradiation time. The decaBDE disappeared relatively quickly over the first 24 hours of light

exposure, after which the concentration remained relatively stable. The accumulation of bromide ion showed an almost linear increase from 12 hours to the end of the 72-hour exposure period. Approximately 30% of the initial decaBDE had disappeared after 72 hours' exposure. The dark controls only showed a slight decrease in concentration over time (~1%), and showed no detectable levels of bromide ion. The different pattern in the kinetics of disappearance of the decaBDE and the appearance of bromide ion indicated that the production of bromide ion continued after the loss of parent compound had slowed, i.e. bromide was being generated from the initial degradation products. The bromine mass balance for the system indicated that about 70% of the total bromine present was accounted for by decaBDE or bromide ion, with the remaining 30% being present as unidentified compounds (which might have been polymeric condensation products). Analysis by HPLC indicated the possible presence of nona- and octaBDEs, but not lower PBDE congeners.

- The fourth series of experiments investigated the solar irradiation of decaBDE adsorbed to quartz tubes containing water, prepared in the same manner as the previous experiment. The results of these tests showed a much more rapid loss of the parent substance than found with the other experiments when humic acid was present. Approximately 71% of the initial decaBDE was lost after 72 hours' irradiation over a 15-day period, and the rate of loss was relatively constant over the entire 72-hour period. Bromide ion was also shown to accumulate at a steady rate over the 72-hour period. Analysis of the dark controls showed that no bromide ion was present and no significant loss of parent substance had occurred. The mass balance indicated that approximately 50% of the total bromine was present as either decaBDE or bromide ion. The identity of the remaining 50% could not be determined with the analytical methods used. Analysis by HPLC indicated the possible presence of nona- and octaBDEs, but not lower PBDE congeners. The HPLC peak areas were lower than for the experiment with humic acid. The difference between the experiments in quartz tubes with and without humic acids can be explained in terms of the humic acids themselves absorbing light and thus attenuating the degradation process.

- The fifth set of experiments investigated the photolysis of decaBDE in a Rayonet Reactor using two 3,000 Å lamps. The exposures were carried out in quartz tubes prepared in a similar way to the experiments above with natural sunlight (each tube contained 0.019 mg of test substance and 2 ml of water). Exposure was via a merry-go-round system which rotated at 5 rpm. Blank controls and dark controls were also run.

  The results from this series showed that decaBDE was rapidly degraded under the conditions used, even though it was present above its water solubility limit. Around 69% of the initial amount of decaBDE had degraded after 60 hours' photolysis. A decline in the amount of decaBDE present in the dark control was also seen over the 60-hour period, but this decline was at a much slower rate than seen in the irradiated samples, and little or no bromide ion was detected in the dark controls. Bromide ion was found to rapidly accumulate in the irradiated samples, but the amount accumulated levelled off after 24 hours' exposure. The bromine mass balance indicated that decaBDE and bromide ion accounted for a substantial proportion of the total bromine present and indicated that once the parent substance had degraded, any transformation by-products or intermediates also degraded quickly. The fraction of bromine as unidentified products was always below 27 %.

- The final series of experiments essentially repeated the fifth series but used four instead of two 3,000 Å lamps as the irradiation source. Each quartz tube contained 0.77 mg of decaBDE and 2 ml of water. The primary objective of this series of experiments was to identify any by-products formed by using a higher initial concentration of decaBDE (significantly above the water solubility limit).

Under these conditions, the substance was found to degrade more slowly than in the previous series of experiments using the Rayonet Reactor, and a significant amount of decaBDE remained even after 240 hours' irradiation. The accumulation of bromide ion followed a linear trend over the 240-hour exposure period. There was an apparent loss of decaBDE in the dark control samples over the same time frame, but this loss was much less than seen in the irradiated samples. The bromine mass balance indicated that decaBDE and bromide ion accounted for a significant proportion of the total bromine present over the course of the experiment, indicating again that once formed any intermediate degradation products from decaBDE themselves degraded quickly. The amount of unidentified bromine never exceeded about 20% of the total throughout the study. HPLC analysis indicated the presence of small amounts of substances with elution times shorter than the octaBDE congeners of a commercial octaBDE product, which may indicate the presence of other lower PBDEs. Unfortunately, no GC/MS analysis of these products was undertaken.

This series of experiments appears to have been well conducted. They demonstrate that photodegradation can occur relatively quickly in environmentally relevant matrices, with the possible formation of nona- and octaBDEs (and tentatively also hepta- and hexaBDEs) over a period of a few days. However, the short duration of exposure to light and limited attempts at characterising degradation products make it impossible to draw any firm conclusions as to the possible extent of formation of these and other products over longer timescales.

e) An in-depth investigation of the photodegradation of decaBDE has been undertaken by Palm et al. (2003). The results of some of these experiments are also reported in da Rosa et al. (2003). Most of the experiments used organic solvents and so are not considered here. However, one experiment was carried out to investigate the aqueous photolysis of decaBDE adsorbed on to silicon dioxide particles. A test solution was prepared by firstly dissolving 40 mg of decaBDE in 1 ml of tetrahydrofuran, to which was added 1 g of silicon dioxide particles (specific surface area 380 m^2/g). The mixture was stirred at 11,000 rpm for five minutes. After this time the solvent was removed and the coated silicon dioxide particles were vacuum dried overnight. The test suspension was then prepared by adding 100 ml of water to 50 mg of decaBDE-coated particles and stirring at 11,000 rpm for 5 minutes. The nominal concentration of decaBDE in the final suspension was 20 mg/l. The freshly prepared suspension was irradiated for 45 minutes with stirring using polychromatic light (λ >280 nm). Around 45% of the substance was found to have degraded by the end of the test. Details of all the degradation products formed during this study were not given in the test report but it was shown that brominated dibenzofurans were formed.

   This study therefore suggests that the primary aqueous photolytic half-life of decaBDE on suspended particles is approximately one hour. Whilst the identity of all the degradation products is unknown, brominated dibenzofurans were formed.

f) Eriksson et al. (2004) studied the photochemical degradation of decaBDE in water (with and without the presence of humic acids) using artificial UV light in the sunlight region. The test substance had a purity of >98%. The experiments were carried out in a cylindrical vessel with a 20 watt fluorescent tube placed longitudinally through the middle. Around 20 ml of a saturated solution of decaBDE in ethanol was transferred to a conical flask (and a solution of humic substances (50 mg in 10 ml of ethanol) was added if being used) and approximately 10 ml of the ethanol was then evaporated. After this the flask was filled with 2 litres of water and heated at 80°C for 1 hour. Once cooled the solution was used directly in the photolysis experiment (the final humic substance concentration would have been around 25 mg/l and traces of ethanol would also likely have been present (i.e. <5 to <10 ml/l; it is not clear how much ethanol would have been lost by heating at 80°C)). Experiments

were carried out in at least duplicate, and the samples were irradiated for 100 minutes (i.e. less than two hours).

The substance was found to photodegrade in water with humic acid, with a first order rate constant for the removal of around 3×10^{-5} s^{-1} (half-life around 6.4 hours). In a parallel experiment using organic solvents, degradation was found to occur by consecutive debromination down to hexaBDEs after 100 minutes' irradiation, and products with less than six bromine atoms were also formed (tentatively identified as brominated dibenzofurans and possibly methoxylated brominated dibenzofurans). The rate of photodegradation also decreased with decreasing degree of bromination, and was also influenced in some cases by the bromine substitution pattern. The experiments in water containing dissolved humic substances gave rise to an almost identical set of products, but with a higher proportion of pentabromodibenzofurans.

The experiments using pure water were reported to be very difficult to carry out, and it is possible that the observed disappearance could have resulted from adsorption to the glass wall since no degradation products were apparent in these samples. The half-life for this removal was around 39 hours.

This study appears to have been well conducted, and shows that phototransformation to hexaBDEs is possible in aqueous systems containing humic substances over a relatively short timescale. However, the laboratory condtions are not directly applicable to the environment, and the short duration of the study means that the relevance of this mechanism over longer timescales could not be established.

g) Gerecke (2006) [ABST] determined the reaction quantum yield for the photodegradation of decaBDE on the clay mineral kaolinite. The photolysis experiments were carried out using natural sunlight at Dübendorf, Switzerland (47°25' N, 8°37' E), at around noon on clear summer days. In the experiments a series of thin solid layers of kaolinite that had been spiked with decaBDE on glass slides were used. Most of the samples were pre-conditioned at 50% relative humidity (dry conditions) prior to exposure, but some experiments were also carried out by adding water to the mineral layer (wet conditions). The temperature in the experiments was controlled by means of a water bath. Experiments investigating the penetration of light into thin layers of kaolinite found that both the absorption and scattering coefficients varied with wavelength in the range 250 to 700 nm. In dry kaolinite, only a very small amount of light was found to penetrate below 50 µm. Thus it was concluded that only decaBDE that is sorbed to particles at the surface is likely to undergo photolysis.

The photolysis experiments using thin layers of spiked kaolinite showed a decrease in decaBDE concentration. The non-exponential decay was thought to reflect the fact that, even in the very thin layers used (around 6 µm), the intensity of light in the layer varied by more than a factor of 2 between the top and bottom of the sample. However, to analyse the data, a first order decay process was assumed. Using this assumption, the degradation half-lives were estimated to be around 76 minutes for dry conditions and 73 minutes for wet conditions. The calculated quantum yield (again assuming a first order decay process) was 0.1 (±50%).

Analysis of the degradation products was also attempted. Under dry conditions, the majority of the degradation products were reported to be lower PBDEs. However, under wet conditions a large proportion of the degradation products were unidentified. No further details of the identities of the degradation products were given in the paper, but the analytical methodology used appears to have investigated mainly hepta-, octa- and nonaBDEs.

h) Ahn et al. (2006a) reported another experiment investigating the photodegradation of decaBDE adsorbed to clay minerals (montmorillonite and kaolinite). Additional experiments involved metal oxides (manganese dioxide (birnessite), iron oxide (ferrihydrite) and aluminium hydroxide) and organic carbon-rich natural sediment as the solid phase (the sediment was collected from the Celery Bog Park, West Lafayette, Indiana, and was a loam sediment, of pH 6.3 and an organic carbon content of 16.4%). The samples were prepared by adding 100 µl of a stock solution of decaBDE in tetrahydrofuran (the concentration of the stock solution was 1.0 g/l) to 250 mg of the solid phase in 15 ml glass culture tubes. The solvent was then removed by air-drying in the dark for 30 hours and each sample in the tube was then homogenised and 500 µl of water was added. The tubes were then sealed and irradiated with natural sunlight or in a photochemical reactor equipped with four 24 W black light-phosphor UV lamps (wavelength range 300-400 nm, maximum light intensity at 350 nm). The experiments using the photochemical reactor were carried out for up to 14 days' exposure. During the exposure, samples were rotated past the light source at 5 rpm. The experiments using natural light were carried out between July and November 2004 in West Lafayette, Indiana, United States (40°26' N, 86°54' W), and exposure was for up to 101 days (the paper indicates that further exposure in November/December did not result in any further degradation of decaBDE).

   During the sunlight exposures, the sample tubes were placed on a wooden board at an angle of 45° and the samples were rotated through 120° once every week. No degradation of decaBDE was observed in any of the dark control samples. Light control samples (prepared in the same way as the exposure samples but without any added solid phase) also showed no direct photodegradation. Degradation was, however, evident when decaBDE adsorbed to the various solids was exposed both to natural sunlight and in the photochemical reactor experiments. The disappearance half-life of decaBDE using the photochemical reactor was estimated to be around 150 days with sediment (longer and shorter half-lives were observed with the other solid phases). The equivalent half-life determined under natural sunlight was 990 days. It should be noted that these half-lives are longer than the actual exposure periods used in the experiments.

   Various lower PBDEs were found to be formed, but only the experiments with kaolinite and montmorillonite produced sufficient amounts of degradation products to allow identification. Degradation products were identified by gas chromatography with an electron capture detector, matching retention times of PBDE standards listed to retention times of product peaks. Unknown congeners were assigned to homologue groups based on relative retention times obtained from the literature. Given the very high likelihood of co-elutions the observed peaks cannot be unequivocally assigned to specific PBDE congeners. Nevertheless, the product distribution was found to be similar in the experiments with both natural sunlight and the photochemical reactor. The products were consistent with a stepwise debromination reaction, initially forming nona-, then octa- and heptaBDE congeners by day 3 (photochemical reactor experiments) or day 14 (sunlight exposure experiments). With increased exposure time, hexa- to tribromoBDEs were also formed.

Discussion

Several studies have shown that decaBDE adsorbed as a thin film on solid surfaces in water can photodegrade relatively quickly. Interpretation of the studies is complicated by the range of light intensities and wavelengths that were used. The most relevant studies are likely to be those that used exposure to natural sunlight. The identity of the products is inconclusive in some studies (e.g. Örn (1997), Jafvert and Hua (2001a and 2001b), Palm et al. (2003) and Gerecke (2006) [ABST]), but the study of Sellström et al. (1998) [ABST] and Tysklind et al. (2001) [ABST] provides good evidence for the formation of hepta- and hexaBDE congeners in freshly spiked sediment following exposure to light over 96 hours under laboratory conditions. It appears that substances other than PBDEs

might also be formed, including brominated dibenzofurans. Further evidence for the formation of hexaBDEs is provided by the studies of Jafvert and Hua (2001b) and Eriksson et al. (2004). Ahn et al. (2006a) found that debromination of decaBDE adsorbed to the minerals kaolinite and montmorillonite was a stepwise reaction, initially forming nona-, then octa- and heptaBDE congeners after 14 days' exposure to sunlight. With increased exposure time, hexa- to tribromoBDEs were also formed.

In contrast, light does not appear to have been a significant factor in the decaBDE transformation observed over 12 days in a recent *in situ* sediment degradation study in a Canadian boreal lake reported in Section 3.1.2.2 (the presence or absence of oxygen seems to have been more relevant). This does not necessarily imply that light is not important over longer timescales.

The environmental behaviour of decaBDE means that the majority of the substance that is released to the aquatic environment will partition to suspended particulate matter and ultimately sediment (see Section 3.2), where it is likely to be immobile. In these matrices only the surface layer is likely to be exposed to light, and in many sediments the amount of light reaching the sediment surface will be low due to light attenuation by water, humic substances and other materials. A number of other mitigating factors can be expected to influence aquatic phototransformation. These include the effects of sorption of decaBDE to colloid particles, and the generally low concentrations and/or less favourable hydrogen donors present in natural waters (Bezares-Cruz et al., 2004). Quenching agents are also likely to be present. It might therefore be expected that only a very small fraction of the total decaBDE present in aquatic environments would have the potential for photodegradation.

In addition, the studies provide little information as to whether the products found at the end are continuing to build up or are decreasing in the system. The effect of multiple or continuous input of decaBDE is not considered. Therefore, although these studies provide some evidence for the potential phototransformation of decaBDE to nona-, octa-, hepta- and hexaBDEs in aquatic environments, they cannot be used to conclude on the extent and rate of their formation in the environment.

3.1.1.2.3 Phototransformation in soil

The registration dossiers have one study summary for this end point based on two references (Sellström et al., 2005 and Söderström et al., 2004). These are summarised below, together with information from additional related articles.

- Sellström et al. (1998) [ABST] and Tysklind et al. (2001) [ABST] both report a study of the photolytic degradation of decaBDE using a variety of media, including sand and soil (the study is summarised in Section 3.1.1.2.2 for sediment, and samples were prepared in the same way, although water was not added). The soil was an agricultural soil from Jyndevad, Denmark, selected as a 'typical' Nordic soil. The removal half-life for decaBDE in the sand experiments was around 35-37 hours using natural sunlight. The corresponding removal half-life in soil was estimated to be 200 hours (Tysklind et al., 2001) [ABST].

  Söderström (2003) and Söderström et al. (2004) provide a more detailed discussion of the products formed in this study. The products were broadly comparable across the different media. Nona-, octa- and heptaBDEs were formed along with lower PBDEs (it was not always possible to identify the exact congeners due to the lack of suitable reference material). 2,2',4,4',5,5'-HexaBDE was found in the experiments using sand (outdoor exposure) and 2,2'4,4',5,6'-hexaBDE was found in all exposures. Below hexaBDEs the mass balance (based on the amounts of lower PBDE congeners found) was low, indicating that other compounds were being formed. Tetra-, penta- and hexabromodibenzofurans were also detected in the sand and soil experiments. The results were interpreted in terms of an initial stepwise debromination process with the formation of nona- to hexaBDEs. Parallel experiments with silica gel as the solid

support produced 2,2',4,4'-tetraBDE, 2,2',4,4'5-pentaBDE and 2,2'4,4',6-pentaBDE in small amounts, but no tetra- or pentabromodibenzofurans. This was explained by the authors in terms of the optimal conditions used in this test (e.g. due to transparency and shape of the silica gel) compared with the conditions used in the more environmentally relevant sand and soil studies (ECB, 2004). The bromodibenzofurans might have been subject to rapid further degradation, for example.

This reliable study provides good evidence for the formation of hepta- and hexaBDE congeners in freshly spiked soil and dry sand following exposure to light over the course of a few days.

- Sellström et al. (2005) investigated the photodegradation of decaBDE in a field sample of a soil collected in Sweden. The soil had been amended with sludge containing the substance between 1978 and 1982. Samples of the soil were placed in glass test tubes and exposed to artificial UV light on a 'rocking/rolling action' apparatus for up to 21 days. Control (dark) samples were also run. Based on analysis of the PBDE congeners present in the soil samples both before and after UV light exposure, no evidence for photolytic breakdown of the parent substance was seen in either the soil as collected from the field (i.e. no distinctive photolytic pattern was evident in the congeners found to be present in the soil, when compared with the known photolytic pattern found in other laboratory studies), or when the soil samples were exposed to UV light in the laboratory (i.e. no change in the congener patterns present were evident with increased time of exposure to UV light). The soil had clearly been aged for a number of years, so the relevance of the results for freshly exposed soil would appear to be limited. In addition, the formation of small quantities of lower PBDEs cannot be ruled out, since these may have been masked by the presence of the same congeners already in the soil.

Discussion

The conclusion in the registration dossiers is that "photolytic degradation of decaBDE was not observed in farm soils exposed by atmospheric deposition, sewage sludge amendment, and/or river flooding. Some of the farm soils last received sludge-amendment more than twenty years prior to measurement. No evidence of microbial degradation was observed."

The evidence base for phototransformation in soil is small, but one study has shown the potential for the formation of hepta- and hexaBDE congeners in soil and sand freshly spiked with decaBDE following exposure to light (Sellström et al. (1998) [ABST], Tysklind et al. (2001) [ABST], Söderström (2003) and Söderström et al. (2004)).

As for aquatic environments (see Section 3.1.1.2.2), the environmental relevance of this degradation mechanism may be limited by sorption to soil particles and subsequent shielding, light attenuation by humic (and other) materials, etc. Increased adsorption to the soil matrix with ageing might be a further factor that limits transformation via this pathway over longer timescales.

3.1.1.2.4 Other abiotic transformation routes

The registration dossiers do not provide any information on this end point.

A number of studies have been conducted to examine the potential for degradation of decaBDE by reducing agents and minerals in the absence of light[7].

[7] Some studies have considered fundamental reaction chemistry. For example, Rahm et al. (2005) investigated the relative susceptibility of PBDEs to hydrolysis reactions by estimating the second-order rate constant for the reaction with sodium methoxide in methanol/N,N-dimethylformamide. DecaBDE underwent rapid nucleophilic

- Keum and Li (2005) observed extensive debromination of decaBDE to lower PBDE congeners including hepta- and hexaBDEs in experiments using water mixed with iron sulphide, sodium sulphide or powdered iron at 30°C over 14 days (the ratio of decaBDE to iron was 1:100,000). The congener profiles were similar for each reactant, but the rate of transformation varied significantly, with the highest rate observed for iron[8]. For example, 2% and 33% of the decaBDE degraded after 14 days in the experiments with iron sulphide and sodium sulphide, respectively, compared with 90% in the experiment with iron. No hydroxylated products were seen.

  Experiments carried out using specific di- to pentaBDE congeners mixed with iron showed that the rate of the debromination reaction decreases as the number of bromine atoms declines, indicating that the PBDE products formed become increasingly more stable. Further analysis of the products from this reaction was reported by Wang et al. (2008), and Li et al. (2007) performed an additional study of the reaction with zero valent iron (both of these stuides are summarised in EA (2009), but are not considered relevant for this report).

- Ahn et al. (2006b) investigated the debromination of decaBDE in test systems containing birnessite (a naturally occurring manganese oxide mineral), using aqueous tetrahydrofuran (THF) as the solvent. One of the experiments involved water only, and this is the only part of this experiment that is considered relevant for this dossier. DecaBDE (0.1 mg) was added to 50 mg of birnessite (specific surface area 27.7 m^2/g) in a 15 ml test tube using THF which was removed by air-drying in the dark for 20 hours. After this time, 5 ml of water were added, and the contents were shaken continuously for up to 24 hours at room temperature in the dark. All experiments were carried out in triplicate. The degradation products were determined at various time points. Little or no degradation was seen in this test.

  The study also included out similar experiments using aqueous 1,2-dihydroxyl benzene (catechol) rather than THF. Catechol is a soil humic acid precursor and tannin component, and can be readily oxidised by birnessite. No significant degradation of decaBDE (initial amount 0.104 µmol) was evident in the presence of 0.003-0.045 mmol of catechol but when the amount of catechol was increased to 46 mmol, degradation was evident although the rate was small (around 10% degradation occurred over 23 days; value read from a graph). The degradation products formed under these conditions were not stated.

Discussion

aromatic substitution, and the rate of reaction of the lower molecular weight PBDEs decreased by about a factor of ten for each loss of a bromine atom, suggesting increasing persistence (tetraBDEs did not react under even the harshest conditions). Granelli et al. (2012) also investigated the reductive transformation of fifteen PBDE congeners using sodium borohydride. Pseudo-first-order reaction rate constants of the transformations were determined by monitoring the disappearance of the investigated congeners. Each PBDE congener was tested in a total of ten replicates which showed a relative standard deviation of 31% or less. The reductions lead primarily to formation of lower molecular weight PBDEs. DecaBDE was approximately three times more susceptible to reductive transformation as the three nonaBDEs. The reactivity of the tested octaBDEs varied from 5% to 24% of the reactivity of decaBDE for BDE-196 and BDE-198, respectively. The reactivity of the heptaBDEs was in the range of the less reactive octaBDEs, except for BDE-181 which was as high as 13% of the reactivity of decaBDE. Although these results cannot be directly translated to environmental transformation rates, they indicate the propensity of PBDEs to undergo reduction/substitution reactions, which suggests a diminishing reaction rate with increasing loss of bromine atoms. (These studies have not been summarised in previous EU risk assessment reports.)

[8] The paper gives most details for the experiments with zerovalent iron. The authors of the paper were contacted and provided further details of the concentrations of PBDEs found using the other two reactants, as discussed in ECB (2007a).

The results of these experiments are interesting but cannot be directly extrapolated to the environment. For example, Keum and Li (2005) used reductant concentrations that are not environmentally realistic (e.g. the levels of iron sulphide were at least one order of magnitude higher than the concentrations typically encountered in freshwater sediments), and powdered iron is not a naturally occurring mineral. In addition, Vangheluwe (2005) pointed out that:

- The tests were conducted at 30°C, which is above the temperature encountered in sediments (the REACH technical guidance assumes a standard temperature of 12°C).
- Iron sulphide is present in the solid phase in anaerobic sediments, and sediment particles tend to be coated with organic carbon (to which the substance would adsorb), which would reduce the area available for reaction.
- The excess of sulphate ions in seawater would act as a competing (and preferred) electron acceptor in marine sediments.

This study is therefore of low relevance to environmental conditions[9]. However, there are numerous similar reductants (e.g. iron-bearing minerals and sulphide ions, etc., some of which may be water-soluble) present in anaerobic conditions in both sediments and soils. These are predicted to be the major environmental compartments to which decaBDE will distribute. There is therefore a possibility that similar reactions might occur in some situations, although it is not possible to estimate the extent or rate of any transformation based on these data.

Ahn et al. (2006a & 2006b) observed slow degradation of decaBDE in the presence of birnessite and catechol, which might possibly be environmentally relevant, but the degradation products are unknown.

3.1.1.3 Summary of abiotic degradation

Hydrolysis is unlikely to be a relevant degradation process in the environment.

DecaBDE is likely to be mainly adsorbed to particulates in the atmosphere. The atmospheric residence time of decaBDE is expected to govern phototransformation potential. This depends on particle size, and will be highest during dry periods, but is expected to be in the order of days at most. Phototransformation to several per cent w/w nonaBDEs might be expected under such conditions. These will ultimately be deposited to sediments and soils. Small amounts of other substances such as octa- and heptaBDEs and brominated dibenzofurans might also be formed in some circumstances, although the amounts are likely to be small.

In aquatic environments, decaBDE has the potential to photodegrade relatively quickly, and nona-, octa-, hepta- and hexaBDE congeners have been observed to be formed in freshly spiked sediment following exposure to light over 96 hours under laboratory conditions. Other substances might also be formed, including brominated dibenzofurans. In practice, only a very small fraction of the total decaBDE present in aquatic environments will be available for photodegradation (due to light attenuation, shielding, etc.) and the extent and rate of phototransformation of decaBDE to hepta- and hexaBDEs under realistic environmental conditions cannot be deduced from the available data. A recent *in situ* sediment degradation study did not reveal any significant influence

[9] Nanoparticulate iron has begun to be investigated for use in the remediation of polluted soils (e.g. Karn et al., 2009). If decaBDE were present in such soils, there is a potential for extensive transformation to lower PBDE congeners, although the reactivity of the iron may be more limited than observed in the laboratory experiments. Depending on the duration of contact with the iron (which may be limited by decaBDE's strong adsorption to soil particles), it is possible that these congeners would themselves ultimately be removed, although the lower congeners appear to be more stable than decaBDE.

of light on the observed degradation, although this test was of relatively short duration (12 days). Ageing might also play a role in reducing the potential for this reaction with time. A similar conclusion can be drawn for soil.

Reaction with reductants (e.g. iron-bearing minerals and sulphide ions, etc., some of which may be water-soluble) present in anaerobic conditions in both sediments and soils is a possible additional abiotic transformation route, but it is not possible to estimate the extent or rate of any transformation based on the available data.

3.1.2 Biodegradation

3.1.2.1 Biodegradation in water

3.1.2.1.1 Estimated data

Estimated biodegradation data are not relevant to this dossier.

3.1.2.1.2 Screening tests

The registration dossiers have one study summary for this end point, which is summarised below.

DecaBDE (100 mg/l) was incubated with activated sludge inoculum (30 mg/l) from mixed sources in Japan over a two week period (equivalent to MITI I test (OECD 301C)). No degradation (as measured by biological oxygen demand) was seen, so the substance is not readily biodegradable (CITI, 1992).

This result reflects the fact that the test concentration was around six orders of magnitude higher than the water solubility limit (which is below 0.1 µg/l at 25°C).

3.1.2.1.3 Simulation tests

No simulation tests for degradation in surface water alone are available.

3.1.2.2 Biodegradation in sediments

The registration dossiers have two study summaries for this end point based on four references (an unspecified study report, 2001; unspecified publication, 2002; Nuck and Federle, 1996[10]; and Orihel et al., 2008 [these are not included in the reference list of this document]). These are summarised below or in Section 3.1.3, together with additional information.

A series of experiments have been conducted in a freshwater lake as part of a Canadian government-funded project. Field work began in 2007 and completed in September 2010. Although a formal manuscript containing full experimental details is unlikely to be available until mid-2012, a summary of the experimental set up and main initial findings has been provided by Orihel et al. (2009) [ABST] and Muir (2011) [ABST], and oral or

[10] This study formed the basis for a test protocol for decaBDE but did not use decaBDE as the test article. It is therefore not cited in the reference list of this document. The unspecified report and publication relate to the study conducted for the purposes of the ESR. This is cited in this report as Schaefer & Flaggs (2001a) and Schaefer et al. (2001) [ABST].

poster presentations have also been made for several scientific audiences (e.g. Orihel et al., 2010a [ABST], 2010b [ABST], 2011a [ABST] & 2011b [ABST]). Further details were provided directly by the researchers on request (Muir & Orihel, 2011; Orihel, 2011). Some or all of the experiments will be reported in the open scientific literature in due course. The available information is outlined below. (This work has not been summarised in previous EU risk assessment reports.)

The studies were performed in the Experimental Lakes Area (ELA)[11], a dedicated research facility located in a sparsely inhabited region of Ontario, Canada (49°39'N 93°43'W; elevation 369 m). The facility consists of 58 small (1 to 84 ha) lakes and their watersheds, and a year-round field station that can accommodate up to 50 researchers. It is relatively unaffected by external human influences and industrial activities; road access is restricted, and public access to the watersheds and study lakes is also controlled (e.g. no public motor boats or fishing is allowed on the study lakes). Lake 240 was chosen for the experiments. This lake is oligotrophic, with sandy littoral sediments of low organic carbon content (loss on ignition was below 3%[12]).

Four experiments were conducted to examine the debromination and fate of decaBDE under natural field conditions. Two experiments involved a mesocosm with invertebrates and fish present, and so are reported in Section 3.1.3. Full experimental details are not yet available, so a reliability marking cannot be assigned to any of the individual studies and the interpretation of some of the findings might be subject to change. However, they are the first studies to investigate transformation of decaBDE in sediment under natural field conditions. It is therefore considered relevant to include summaries in this dossier at this stage (if further details emerge during the public consultation period, they can be added in due course). The two studies that are explicitly related to sediment degradation are described below:

i) ***Experiment A***: The objective of this experiment was to quantify and compare the rates of *in situ* debromination in littoral and profundal sediments of Lake 240 (so that any implications from the positioning of the mesocosms in the littoral zone could be investigated). Sediment cores were collected from the littoral (depth (z) = 1.5 m) and profundal (z = 8.0 m) zones in 60 cm-long acrylic tubes. A small sub-sample was freeze dried and spiked in a vial with 80 µl of an acetone solution of ^{13}C-labelled decaBDE (concentration 25 µg/ml) (obtained from Wellington Laboratories as a solution in toluene, with an isotopic purity >99%). The solvent was allowed to evaporate in a dark fume cupboard overnight, and the spiked sub-sample was then poured into the top of the water surface of each tube in dim light conditions, to provide a dose of 2.0 µg of ^{13}C-labelled decaBDE. Sixteen littoral and three profundal sediment cores were incubated in the littoral zone of the lake (z = 1.5 m); three littoral and sixteen profundal cores were incubated in the profundal zone (z = 8.0 m). The cores were held in vessels in a stabilised frame ("benthic lander cradle") at the bottom of the lake, tethered to a marker buoy. The core tube tops were left open to allow the natural sedimentation of particles from the overlying water column and the diffusion of solutes into the core tube. Incubation took place under natural conditions over 30 days. The surface (top 1 cm) sediment layer of each core was sampled for PBDE determination at 0, 1, 3, 7, 10, 16, and 30 days.

   At the end of the exposure period, wet sediment samples (~5 g) from each treatment were mixed with Hydromatrix and the PBDEs extracted and analysed using GC-electron capture negative ion MS (GC-ECNI/MS). Forty-five PBDEs were monitored at m/z 79/81 except for ^{13}C-labelled decaBDE which was monitored at m/z

[11] See http://www.dfo-mpo.gc.ca/regions/central/pub/ela-rle/index-eng.htm and http://www.experimentallakesarea.ca/ELA_Website.html

[12] This is a surrogate measure of total organic carbon content in bulk sediment. A periphyton floc rests on the surface of littoral sediments, which has a higher organic carbon content than the bulk sediment itself.

493/495 and native decaBDE at m/z 487/485. Results were calculated as ng/g dry weight and blank subtracted using the method blank for each batch. Depending on the congener, limits of detection varied between 0.02 and 0.2 ng/g dw.

Preliminary results based on low-resolution analyses were reported in Orihel et al. (2011b) [ABST]. DecaBDE levels (expressed as the percentage of total PBDEs) decreased, on average, by 2% between day 0 and 30, but the linear change over time was not statistically significant due to the high variability observed over the course of the test. There was no difference in decaBDE concentrations among the four treatment groups on day 30. Concentrations of certain nona- and octaBDEs in littoral sediments were above background levels after 30 days, under both littoral and profundal incubation. Concentrations of most lower molecular weight PBDEs in littoral sediments were generally not elevated after 30 days, with a few exceptions (e.g. BDE-99, -100, -28, -33 & -32).

ii) ***Experiment B***: The objective was to examine *in situ* debromination in lake sediments under four different incubation conditions (oxic/light; oxic/dark; anoxic/light; anoxic/dark). Twenty-eight littoral sediment cores (z = 1.5 m) were collected from Lake 240 in 30 cm-long acrylic tubes. Twenty-four of the tubes were each dosed with 2.0 µg of ^{13}C-labelled decaBDE in the same way as Experiment A. The remaining four cores served as controls. Each treatment used six test cores and one control core, and all were incubated in the shallow littoral zone of the lake for up to 12 days (the oxic treatments in one benthic lander cradle and the anoxic treatments in another close by). Oxic conditions were maintained by bubbling the overlying water in the core tube continuously with filtered air (using an aquarium pump). Anoxic conditions were created by bubbling the overlying water in the core tube with nitrogen for approximately 15 minutes, followed by sealing with perifilm. Dark treatments were created by covering the tubes with aluminium foil (light treatments were left uncovered). Three test cores were destructively sacrificed from each treatment on day 6, with the remainder (including controls) sacrificed on day 12.

The temperature of the cores varied diurnally between about 11 and 17°C (values read from a graph). Light intensity and dissolved oxygen levels were successfully manipulated in the four treatments. Peak light intensity in the light treatments varied between about 15,000 and 2,000 lux. Dissolved oxygen concentrations were measured on days 0, 6 and 12, and ranged between about 8 and 11 mg/l in the oxic treatments, remained at about 0.25 mg/l in the anoxic/dark treatment and rose from 0.25 mg/l initially to 4 mg/l at the end of the test in the anoxic/light treatments (all values read from a graph) (the latter value is consistent with dissolved oxygen levels in profundal sediments, which are typically at 1-3 mg/l).

At the end of the exposure period, wet sediment samples (~5 g) from each treatment were extracted and analysed for PBDEs in the same way as Experiment A. Results were calculated as ng/g dry weight and blank subtracted using the method blank for each batch. Depending on the congener, limits of detection varied between 0.02 and 0.2 ng/g dw.

Preliminary results (see Figure 3) show that the concentrations of ^{13}C-labelled decaBDE in spiked sediment cores on day 12 were approximately twenty to fifty times higher than background levels of the substance in Lake 240 sediments. Debromination to nonaBDEs occurred under all treatment conditions. Bromine atoms were preferentially removed at the *meta*- and *para*- positions. Debromination to octaBDEs was evident, mainly under anoxic conditions. Concentrations of heptaBDEs were either below the limit of detection or similar to background levels. Concentrations of hexaBDEs were mostly below the limits of detection.

Figure 3: Preliminary results of the *in situ* debromination of decaBDE under different conditions after 12 days (Experiment B)

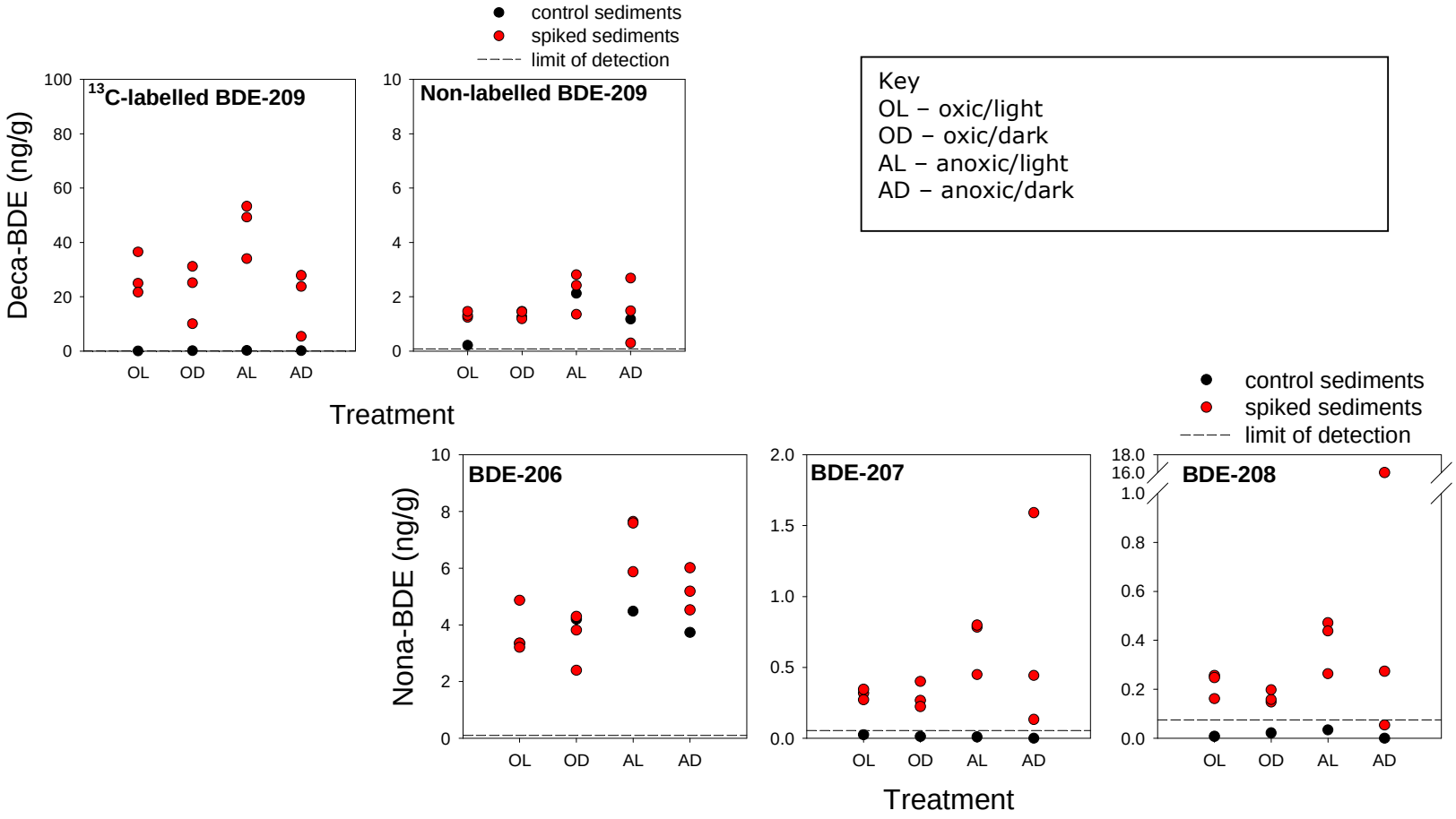


Figure 3 (continued)

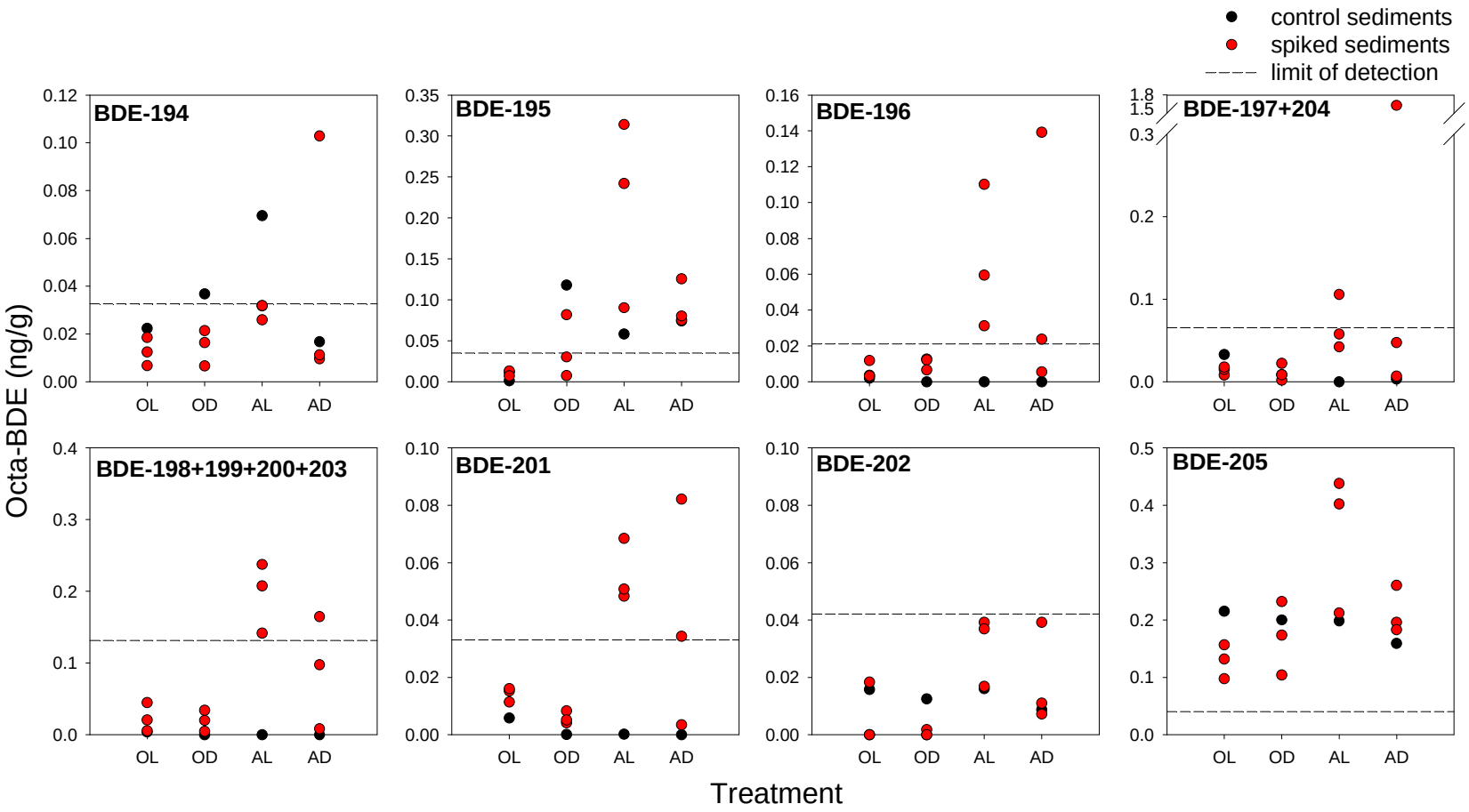


Figure 3 (continued)

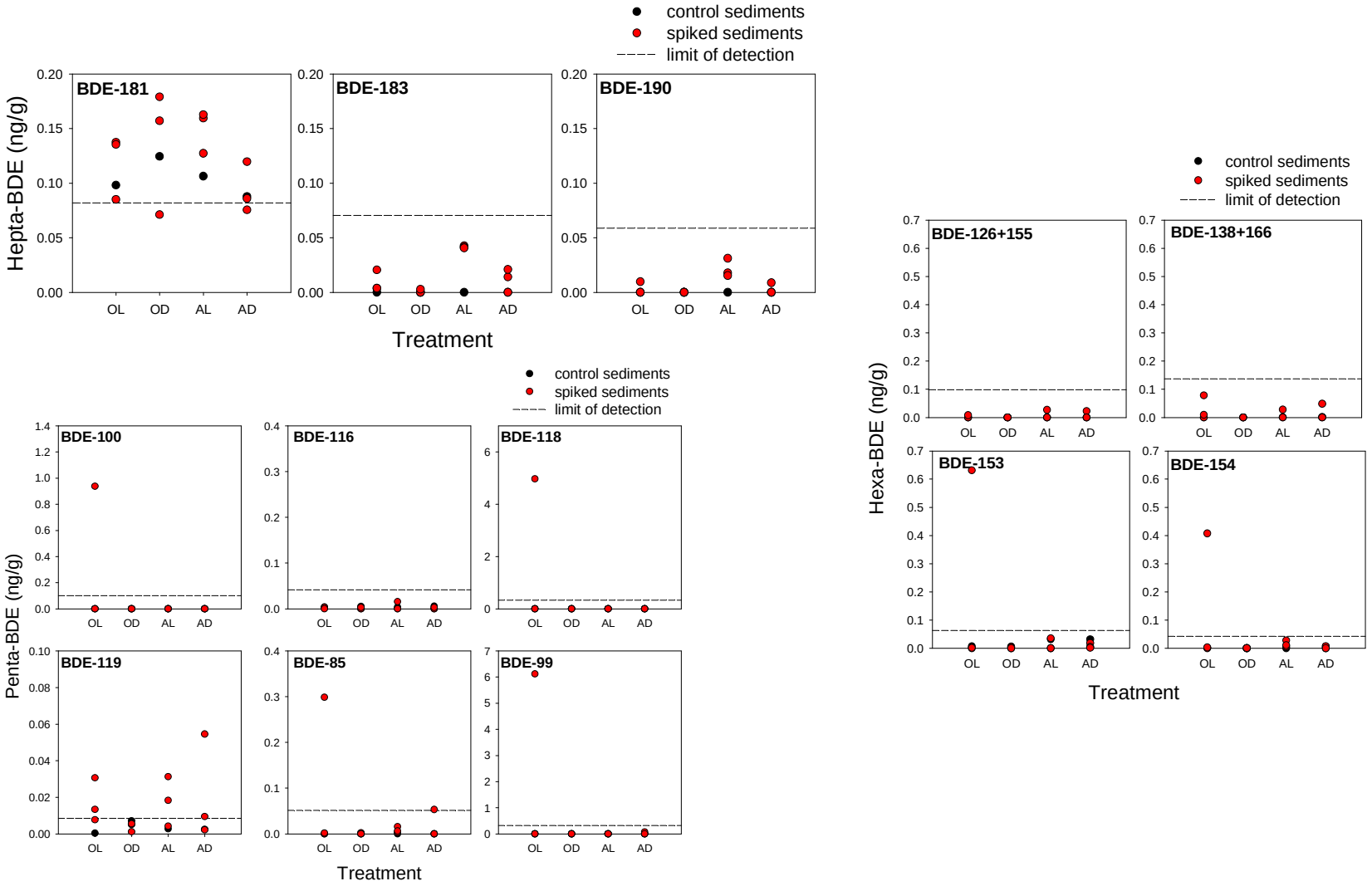
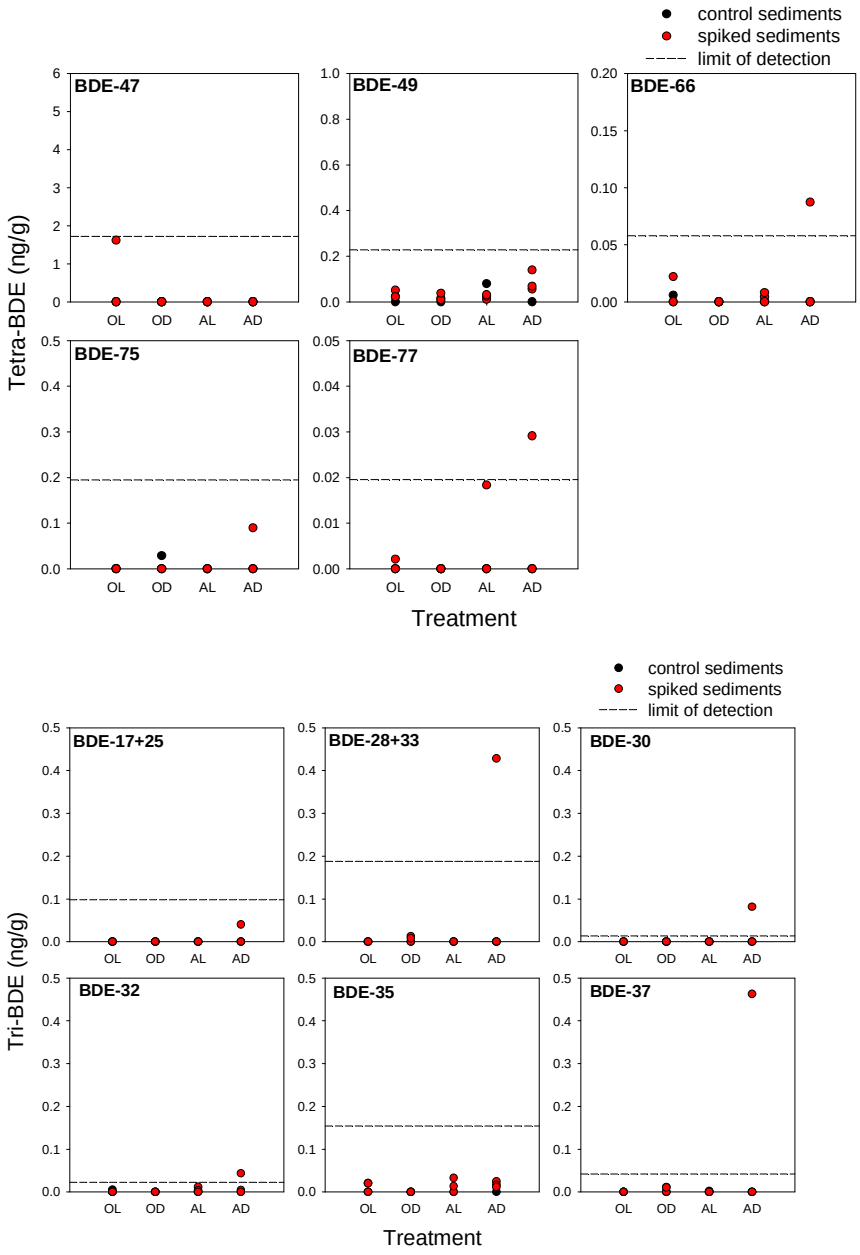


Figure 3 (continued)



One pentaBDE congener (BDE-119) was detected in some treatments, but not in the control sediments; levels of other pentaBDEs were mostly below the limits of detection. Concentrations of tetra- and triBDEs were mostly below the limits of detection, but formation of some diBDE congeners was observed in the oxic/light treatment. Light extinction was significant, with only 10-20% of surface light reaching littoral sediments at a depth of two metres.

Discussion

The two *in situ* sediment degradation studies were performed to aid interpretation of the findings from the mesocosm studies reported in Section 3.1.3. Storage of the cores in the lake allowed degradation to be followed under environmentally realistic abiotic conditions (e.g. natural variation in light and temperature, etc.). In the experiment that investigated the role of light and oxygen, preliminary results suggest that a small percentage of the decaBDE dose added to the sediment cores debrominated under all treatment conditions following incubation in the lake for 12 days (i.e. less than two weeks). The extent and pathway of debromination depended on the incubation conditions: an increase in octaBDE concentration was detected particularly under anoxic conditions, with some lower molecular weight PBDE congeners also apparent in some of the treatments. Similar observations were made in the 30-day experiment, with the apparent formation of nona- and octaBDEs. The absence of significant inputs from other PBDE sources (i.e. commercial penta- and octaBDE products) implies that the findings should relate solely to the input of decaBDE. However, further analysis of a selection of retained samples using high resolution GC/MS (US EPA method 1614) is planned to unequivocally identify ^{13}C-PBDEs (which would prove decaBDE to be the source), since it cannot be excluded that the results reflect existing sediment contamination[13].

The short duration of the experiments means that they do not represent steady-state conditions, but rather give an indication of the rate and extent of initial debromination. Degradation might not occur in a linear way, so it is not currently possible to extrapolate these findings over longer timescales.

Other studies

A large number of other studies have been performed that are relevant to sediment degradation. These are summarised below. Since there are a number of factors that limit their extrapolation to environmental conditions, formal robust study summaries have not been produced for the purposes of this dossier.

1) Qiu et al. (2011) used alternate carbon substrates to enrich PBDE-debrominating microbial consortia collected from sediments. (This study has not been summarised in previous EU risk assessment reports.) Sediment samples were collected from the Lianjiang River at Guiyu, China, at a depth of 5–15 cm, and stored at 4°C. The samples were enriched with vitamins and minerals, 0.2 g/l of yeast extract and 10 mM of an electron donor (either methanol, ethanol, acetate, lactate or pyruvate). 10 µM of a commercial decaBDE product (purity > 98%) in dichloromethane (250 ml) was added to a bottle and evaporated in darkness. Sediment (20 g wet weight) and 150 ml of medium were then added to each glass bottle, which was purged with nitrogen gas for 5–10 minutes, and incubated at 30°C in anaerobic gloveboxes

[13] Even though this locality has a low level of anthropogenic activity, low concentrations of decaBDE and some lower PBDE congeners were present in the lake before the introduction of test material. The researchers believe that these occur as a result of atmospheric deposition.

without shaking for 90 days. The culture medium was exchanged every two weeks. All experiments were conducted in duplicate.

To determine the debromination rate, 2 ml of the culture medium were sampled every 2 weeks. Pre-treatment to remove organic matter and heavy metals was performed before analysis with an ion chromatograph (IC) for bromide. Sediment samples were freeze-dried, ground, and homogenized before extraction and analysis for 21 PBDE congeners using gas chromatograph-mass spectrometery.

The concentration of bromide ion increased from 100 to 500 µg/l over 90 days. No obvious increase in the concentration of bromide ion was detected in the control. The addition of exogenous electron donors in the medium did not enhance the debromination of PBDEs, since the enriched microbial consortium from sediment without any electron donor added produced more bromide ion than those with additional electron donors. The results indicate that most of the microorganisms in the sediment involved in PBDE debromination were oligotrophic and the amount of carbon source in sediment was enough to support the PBDE debromination.

DecaBDE was debrominated to lower molecular weight PBDE congeners by microorganism over 90 days. Differences in the PBDE profile were observed among the consortia enriched on different electron donors. BDE-154 was only observed in the methanol, ethanol, and acetate enrichments. The percentage of decaBDE was decreased by 12% (methanol), 11% (ethanol), 8% (acetate), 9% (lactate), 5% (pyruvate), and 11% (no electron donors) after 90 days of incubation (compared to the initial profile). The relative abundances of most nona-, octa- and hexaBDEs increased by the same proportion as decaBDE loss, and their formation was also inhibited by the presence of electron donors.

PCR-denaturing gradient gel electrophoresis revealed significant shifts in the microbial community structure among different treatments. The consortium enriched with methanol was similar to ethanol, but differed from acetate, lactate and pyruvate, indicating that electron donors have different effects on the growth of microbes presented in the sediment. A total of 19 dominant bands were excised from gels, and their nucleotide sequences were determined and compared with 16S rRNA gene databases. Biodegradation rate was found to be correlated with the abundance of *Pseudomonas* spp. and related species. No *Dehalococcoides* species were detected.

Although the temperature and other test parameters do not represent environmental conditions, this study provides strong evidence that micro-organisms present in natural sediments are capable of debrominating decaBDE to at least hexaBDEs.

2) Lee and He (2010) established microcosms with soils and sediments from 28 locations (from China, Singapore and the USA) to determine their debromination potential with a commercial octaBDE product consisting of hexa- to nonaBDEs. (This study has not been summarised in previous EU risk assessment reports.) Collected samples were transported to the laboratory at ambient temperature. Within one week, microcosms were established aseptically in a Bactron anaerobic chamber, in which 5 g portions of collected samples were added to 60 ml serum bottles containing 30 ml of bicarbonate-buffered mineral salts medium. Each sample was spiked with pyruvate, lactate or acetate (to investigate the influence of different carbon sources) in duplicate bottles for each treatment. The media were reduced by adding l-cysteine and sodium sulfide, followed by addition of vitamin solutions. Hydrogen gas was added to acetate-containing microcosms, ensuring minimal change in the pressure in the bottles. All the bottles were crimp sealed with butyl rubber septa. The test substance (0.05 g) was dissolved in 10 ml solvent (either trichloroethene (TCE) as an electron acceptor, or nonane as a relatively inert solvent

in comparison), and a small volume of the solution added to the samples and controls. The amount of TCE solution added to each bottle was one half of that of the nonane solution due to concerns about the toxic effects of TCE on the microbes. All the sample bottles were incubated in an upright position (to minimize sorption to the butyl rubber septa) at 30°C without agitation in the dark for 60 days. Biweekly, samples were taken from each microcosm and PBDEs extracted and analysed using GC/MS (selected ion monitoring in the electron impact ionization mode). To minimize sorption effects on the quantification of PBDEs, the active microcosms (one microcosm from each location) and autoclaved controls were transferred once to fresh medium prior to extraction and analysis.

Debromination products were not observed in autoclaved controls throughout the experiment. Debromination occurred in microcosms containing samples from 20 of the 28 locations when they were spiked with octaBDE/TCE. Debromination products began to appear after one month. By day 60, penta- and tetraBDEs had increased in most samples. Tri- and diBDEs, were formed in 12 and 4 of the 20 active microcosms, respectively. These daughter compounds were not detected in the original soil or sediment samples, confirming that they were indeed debromination products and not due to historical contamination. TetraBDEs accounted for 50% of the total debromination products in all active microcosms.

Although generally less extensive, debromination was also observed in microcosms containing samples from 11 of the 28 locations when they were spiked with octaBDE/nonane. After 60 days, hexaBDEs were detected in all active samples, while penta- and tetraBDEs appeared in 5 of the 11 samples. Debromination of some congeners (e.g. heptaBDE (BDE-183) and octaBDE (BDE-203)) was more evident when nonane was used as the carrier solvent. Microcosms amended with hydrogen gas and octaBDE/nonane were able to debrominate a wider range of substrate congeners (nona-, octa-, and heptaBDEs) than the other microcosms (in which only hepta- and hexaBDE debromination was observed). The microcosms which exhibited high debromination rates with octaBDE/TCE did not exhibit debromination with octaBDE/nonane, and vice versa.

In one sediment-free culture amended with the octaBDE in nonane (containing 45 nM nonaBDE, 181 nM octaBDEs, 294 nM heptaBDE, and 19 nM hexaBDE) there was extensive debromination of the parent compounds, which produced hexaBDE (56 nM), pentaBDEs (124 nM), and tetraBDEs (150 nM) within 42 days, possibly by a metabolic process. With the generation of debromination products, significant amounts of the substrate congeners were removed (46% removal of nonaBDE; 57% removal of octaBDE; 75% removal of heptaBDE; and 55% removal of hexaBDE). No debromination beyond tetraBDEs was detected even after an extended incubation period (8 months) and amendment with additional hydrogen gas.

The carbon source was also found to influence congener patterns.

rRNA gene-based analysis revealed that *Dehalococcoides* species were present in 11 of 14 active microcosms. However, unknown debrominating species in some of the microcosms debrominated the octaBDE mixture in the absence of added halogenated electron acceptors (i.e.TCE).

This reliable study indicates that micro-organisms dwelling in natural environments from a range of locations are able to debrominate PBDEs anaerobically in a matter of weeks, with the formation of hexaBDEs even in circumstances where no electron acceptors were added. The temperature of the study is not typical of sediments, but this may simply mean that the reaction will be slower under normal conditions.

3) Deng et al. (2011) isolated *Lysinibacillus fusiformis* strain DB-1, an aerobic bacterium, from Chinese riverine sediments that were contaminated with PBDEs. (This study has not been summarised in previous EU risk assessment reports.) A single colony was inoculated in 50 ml of growth medium containing 300 mg decaBDE and 20 mM sodium lactate in a flask for aerobic cultivation on a rotary shaker at 150 rpm. Samples were prepared in duplicate and sterile samples were used as a control. The flasks were sealed and all the cultures were wrapped with aluminum foil during the experimental period to avoid photo-degradation. DB-1 was found to efficiently transform decaBDE – in liquid cultures using a nominal initial decaBDE concentration of 6 mg/l, free bromide accumulated to 1,220 µg/l after 72 hours' aerobic incubation at 30 °C with lactate as the carbon source. PBDEs appear to have been detected using gas chromatography-mass spectrometry operated in electron impact ionization mode. No details of any analytical standards are provided, but it was 'deduced' that octa- and heptaBDEs were formed. The resting cell activity tests showed that this was an aerobic process. [14]
4) Schaefer & Flaggs (2001a) performed a simulation test with an anaerobic river sediment treated with decaBDE. The test substance was a mixture of unlabelled decaBDE (supplied as a composite sample from three manufacturers; purity 97.4%, with 2.5% nonaBDE and 0.04% octaBDE) and ^{14}C-labelled decaBDE (radiochemical purity 96.8%). Sediment and accompanying overlying surface water was collected from the Schuykill River, Valley Forge, Pennsylvania, USA. The sediment was described as a loam, with a composition of 50% sand, 29% silt and 21% clay, a redox potential of -284 mV, an average moisture content of 26%, a pH of 6.3 and an organic matter content of 1.4%. A 0.2 mg/l resazurin solution was prepared using the collected overlying surface water.

   Test vessels consisted of 500 ml bottles containing 300 ml of the sediment prepared in an anaerobic chamber. The sediment was carefully added to the bottles to maintain the sediment column structure. The ^{14}C-labelled test substance was added to dry sediment as a solution in tetrahydrofuran and allowed to stand for 24 hours for the solvent to evaporate. The unlabelled test substance was added by direct weight addition to the surface of the sediment (and mixed into the top 2.5 cm layer) to give the desired nominal concentrations of 5 and 500 mg/kg dry weight (three replicate chambers for each concentration). Approximately 10 ml of the resazurin solution was then added to the sediment system. The test vessels were then incubated in an anaerobic chamber in the dark at 22^{o}C for 32 weeks (224 days or 8 months). Six further treatment groups at 5 mg/kg and 500 mg/kg were run to allow metabolite concentrations to be determined at the start and end of the test. ^{14}C-labelled glucose was also tested in the same system at a concentration of 5 mg/kg in duplicate chambers as a positive control.

   The headspaces in the test vessels were continually purged with nitrogen and the production of $^{14}CO_2$ and $^{14}CH_4$ was measured over the 32-week incubation period. At the end of the incubation period, samples from each treatment group were analysed

[14] Yen et al. (2009) investigated the degradation of tetra-, penta- and hexaBDEs (BDE-47, -99, -100, -153 and -154) by anaerobic bacterial mixed cultures isolated from Taiwanese river sediment. Enriched bacterial cultures were mixed with 0.1 µg/ml of PBDEs and incubated at 30 °C for 70 days. PBDE concentrations were determined by gas chromatography with an electron capture detector, and bacterial community changes were analyzed by denaturing gradient gel electrophoresis. Less than 20% of the PBDEs were degraded by the end of the study in all samples except for BDE-47 from one sediment. In that culture, BDE-47 was found to have degraded to a negligible level following an initial lag phase of 42 days (this result was confirmed by a repeat experiment). Addition of the PBDEs changed the composition of the bacterial communities, and in the case of BDE-153 or -154, the change was immediate and irreversible. Although not directly relevant to this dossier, this study again shows that microbial communities present in sediments have the capacity for degrading PBDEs.

for decaBDE and the presence of any degradation products by a HPLC method using both UV and radiometric detection. In addition, a more detailed GC/MS analysis was carried out on several sediment samples at day 0 and week 32 of the experiment to see if trace amounts of lower PBDE congeners were formed (Schaefer and Flaggs, 2001b). The samples for trace analysis were randomly selected and so the replicates analysed at week 32 were not necessarily the same as those analysed at day 0.

The results are shown in Table 6 and Table 7.

Table 6: Mass balance for the anaerobic degradation of ^{14}C-labelled decaBDE

Treatment (nominal)	**Mass balance at week 32**			
	% as $^{14}CO_2$	**% as $^{14}CH_4$**	**% ^{14}C in solids**	**Total % recovery of ^{14}C**
5 mg/kg dw decaBDE	0.4±0.04	0.4±0.04	129.9±24.1	130.9±24.1
500 mg/kg dw decaBDE	0.4±0.03	0.4±0.06	122.5±7.9	123.3±7.9
Positive control (5 mg/kg dw glucose)	67.2±2.1	18.1±1.1	9.5±4.9	94.9±1.8

For the positive control, an average of 95% of the total radioactivity added as glucose was recovered from the system, with 85% converted to $^{14}CO_2$ and $^{14}CH_4$ and 10% associated with the sediment phase. This indicates that the sample pre-treatment methods (e.g. use of tetrahydrofuran solvent) had little effect on the viability of the microbial community present.

Effectively no decaBDE mineralization was detected based on gas evolution in the three main replicate test vessels at either concentration (<1% of the total radioactivity added was found as $^{14}CO_2$ and $^{14}CH_4$). Parent compound analysis (mean of seven replicate samples) indicated that the concentrations of decaBDE in the nominal 5 mg/kg treatment were 6.64±0.70 mg/kg at day 0 and 6.51±2.15 mg/kg at week 32[15]. Similarly, the measured concentrations of decaBDE in the nominal 500 mg/kg treatment were 543±77 mg/kg at day 0 and 612±158 mg/kg at week 32. The differences in concentration between day 0 and week 32 were not statistically significant. Sediment composition was found to account for some of the variability in the measured concentrations, with sediments containing a greater number of stones leading to a higher variability between replicate measurements of concentration. The HPLC chromatographic profiles also indicated that traces of some ^{14}C-labelled components with shorter retention times than decaBDE were present in some of the 32-week samples in the 5 mg/kg dw treatment group. However, similar components also appeared to be present in the stock solution of the ^{14}C-labelled decaBDE test material used in the study.

[15] The ± figures are taken directly from the study report, although it is not clear whether they represent 95% confidence intervals or another measure of variance (e.g. standard error).

Table 7: Results of trace analysis for PBDE congeners from the anaerobic degradation of decaBDE (Schaefer & Flaggs, 2001b)

Congeners	Concentration[b] in laboratory blanks (ng/kg dw)						Concentration[b] in experimental samples (ng/kg dw)					
	A	B	C	D	E	F	Control sediment sample	Day 0 sample (5 mg/kg treatment)	Day 0 sample (500 mg/kg treatment)	Week 32 sample[a] (5 mg/kg treatment)	Week 32 sample (5 mg/kg treatment)	Week 32 sample (500 mg/kg treatment)
2-MonoBDE	<2,470	<2,320	<2,290	<2,230	<3,050	<3,350	<1,480	<1,760	<1,900	<2,180 and <6,860	<2,550	<1,470
3-MonoBDE	<1,730	<1,630	<1,610	<1,470	<2,010	<2,200	<975	<1,160	<1,250	<1,530 and <4,810	<1,680	<971
4-MonoBDE	<1,590	<1,490	<1,470	<1,350	<1,850	<2,030	<899	<1,060	<1,150	<1,400 and <4,410	<1,550	<895
2,4-DiBDE	<15.3	<13.8	<13.6	<21.8	<19.4	<27.3	<36.6	<34.4	66.6	<16.1 and <21.8	262	267
2,4'DiBDE	50.8	50.9	16.2	<16.7	<14.9	<20.9	<28.0	<26.3	<18.4	49.4 and 24.2	87.1	80.3
2,6-DiBDE	<14.1	<12.7	<12.5	<20.5	<18.2	<25.6	<34.3	<32.3	<22.5	<14.8 and <20.1	<22.0	<18.7
3,3'-DiBDE												
3,4-DiBDE	<9.98	<8.96	<8.82	14.3	<11.5	<16.2	<21.7	<20.4	<14.2	<10.5 and <14.2	<13.9	22.9
3,4'-DiBDE	<11.0	<9.87	<9.72	<16.4	<14.6	<20.5	<27.5	<25.9	<18.0	<11.5 and <15.6	<17.6	46.0
,4'-DiBDE	<9.23	<8.29	<8.16	<13.1	<11.7	<16.4	<22.0	<20.7	16.6	10.2 and 13.2	60.2	59.6
2,2',4-TriBDE	42.3	79.4	79.0	<45.9	<31.3	<39.8	119	47.4	74.7	88.8 and 114	398	404
2,3',4-TriBDE	<30.5	<44.1	<31.0	<56.7	<38.6	<49.0	<52.0	<45.2	<39.6	<51.5 and <63.4	60.6	36.3

Congeners	Concentration[b] in laboratory blanks (ng/kg dw)						Concentration[b] in experimental samples (ng/kg dw)					
	A	B	C	D	E	F	Control sediment sample	Day 0 sample (5 mg/kg treatment)	Day 0 sample (500 mg/kg treatment)	Week 32 sample[a] (5 mg/kg treatment)	Week 32 sample (5 mg/kg treatment)	Week 32 sample (500 mg/kg treatment)
,4,4′-TriBDE	109	141	133	<57.8	45.8	<50.0	117	74.3	167	167 and 129	239	250
2,4,6-TriBDE	<28.9	<41.8	<29.4	<53.4	<36.4	<46.2	<49.0	<42.6	<37.3	<48.8 and <60.1	<40.8	<29.0
2,4′,6-TriBDE	<26.7	<38.7	<27.2	<48.8	<33.3	<42.3	<44.8	<39.0	<34.1	<45.1 and <55.6	<37.3	<26.5
2′,3,4-TriBDE												
3,3′,4-TriBDE	<18.8	<27.2	<19.1	<34.1	<23.2	<29.5	<31.3	<27.2	<23.8	<31.7 and <39.1	<26.0	25.0
3,4,4′-TriBDE	<25.2	<36.5	<25.7	<44.6	<30.4	<38.6	<40.9	<35.6	<31.2	<42.7 and <52.5	<34.1	<24.2
2,2′,4,4′-TetraBDE	394	467	403	259	209	224	3,690	1,380	1,600	996 and 1,200	5,290	4,080
2,2′,4,5′-TetraBDE	<21.6	<33.7	30.9	<26.4	<27.4	<19.3	305	95.6	129	122 and 120	1,020	804
2,3′,4,4′-TetraBDE	74.8	114	113	<32.9	<34.1	<24.0	<80.3	<107	<41.5	138 and 192	227	277
2,3′,4′,6-TetraBDE	<19.5	<30.5	<27.2	<26.1	<27.1	<19.1	<63.8	<84.8	<32.9	<24.8 and 31.9	85.3	139
2,4,4′,6-TetraBDE	26.2	99.6	91.1	<21.5	<22.3	<15.7	<52.5	<69.8	<27.1	102 and 92.5	<19.4	33.1
3,3′4,4′-TetraBDE	<15.8	<24.7	<22.1	<18.3	<19.0	<13.4	<44.7	<59.4	<23.1	<20.1 and <23.5	<16.5	<11.3
2,2′,3,4,4′-PentaBDE	79.5	196	97.4	<42.5	55.9	32.0	225	<141	<154	243 and 152	314	361
,2′,4,4′,5-PentaBDE	331	482	417	188	135	233	4,650	1,910	2,460	1,470 and 1,120	6,760	5,130

Congeners	Concentration[b] in laboratory blanks (ng/kg dw)						Concentration[b] in experimental samples (ng/kg dw)					
	A	B	C	D	E	F	Control sediment sample	Day 0 sample (5 mg/kg treatment)	Day 0 sample (500 mg/kg treatment)	Week 32 sample[a] (5 mg/kg treatment)	Week 32 sample (5 mg/kg treatment)	Week 32 sample (500 mg/kg treatment)
2,2′,4,4′,6-PentaBDE	146	247	159	33.5	<21.9	51.0	1,000	342	363	372 and 294	1,270	992
2,3,3′,4,4′-PentaBDE	<99.3	<91.1	80.0	<64.8	<72.2	<46.9	<343	<216	<235	<83.9 and <120	<19.1	459
2,3,4,5,6-PentaBDE	<104	<95.3	<49.3	<66.8	<74.4	<48.3	<353	<222	<242	<87.7 and <126	350	1,120
2,3′,4,4′,6-PentaBDE	<52.1	<47.8	<24.7	<33.6	<37.4	<24.3	<178	<112	<122	<44.0 and <63.2	<98.8	235
3,3′,4,4′,5-PentaBDE	<37.5	<34.4	<17.8	<25.9	<28.8	<18.7	<137	<86.1	<93.6	37.8 and <45.5	<76.1	<87.8
2,2′,3,4,4′,5′-HexaBDE	<114	174	<79.0	<122	<142	<114	<432	<426	1,620	244 and <153	<296	2,080
2,2′,3,4,4′,6′-HexaBDE	<68.9	<65.2	<47.7	<68.2	<79.2	<63.4	<241	<237	345	<78.4 and <92.7	<165	1,710
2,2′,4,4′,5,5′-HexaBDE	199	232	160	<110	<127	<102	834	616	11,100	622 and 859	1,530	15,900
2,2′,4,4′,5,6′-HexaBDE	91.7	169	159	75.5	<64.3	<51.4	483	250	1,670	471 and 670	824	2,790
2,2′,4,4′,6,6′-HexaBDE	<36.7	<34.7	<25.4	<39.0	<45.3	<36.3	<138	<136	<106	<41.8 and 80.5	124	<51.1
2,2′,3,4,4′,5,6-HeptaBDE	<98.2	<146	<127	<153	<107	<146	<381	<687	2,020	<517 and <511	<483	11,100
2,2′,3,4,4′,5′,6-HeptaBDE	<62.2	<92.5	<80.7	<115	<128	<83.4	721	2,050	47,000	1,830 and 1,490	1,740	67,700
2,3,3′,4,4′,5,6-HeptaBDE	<127	<189	<165	<204	<143	<193	<506	917	7,530	<670 and <663	<642	31,900
2,2′,3,3′,4,4′,5,5′,6-NonaBDE	<173	<208	<188	<393	<384	<240	2,160	52,200	3,950,000	47,000 and 47,700	52,000	5,430,000

Congeners	Concentration[b] in laboratory blanks (ng/kg dw)						Concentration[b] in experimental samples (ng/kg dw)					
	A	B	C	D	E	F	Control sediment sample	Day 0 sample (5 mg/kg treatment)	Day 0 sample (500 mg/kg treatment)	Week 32 sample[a] (5 mg/kg treatment)	Week 32 sample (5 mg/kg treatment)	Week 32 sample (500 mg/kg treatment)
2,2′,3,3′,4,4′,5,6,6′-NonaBDE	<173	<208	<188	<393	<384	<240	1,810	52,200	1,350,000	45,400 and 46,900	51,400	2,590,000
2,2′,3,3′,4,5,5′,6,6′-NonaBDE	<173	<208	<188	<393	<384	<240	1,220	26,800	443,000	24,300 and 22,400	25,200	930,000
DecaBDE	<4,560	<3,860	<7,880	<16,100	<12,600	<13,500	142,000	not analysed	not analysed	not analysed	not analysed	not analysed

Notes: a) Duplicate analyses of the same sample.

b) Concentrations given as less than values indicate that the congener was not detected at the limit of detection.

Trace analysis for twenty-one tetra- to heptaBDE congeners was performed on a composite sample from the six additional treatment vessels at both concentrations using high resolution GC/MS. The results were as follows:

a) In the 500 mg/kg treatment, the levels found at week 32 were consistently higher than the concentrations found on day 0 (see Table 8), and it appears that the sum of tetra- to heptaBDE concentrations roughly doubled from around 75 to 150 µg/kg dry weight over this period. For some congeners, the number of moles increased by more than a factor of five. OctaBDE congeners were not included in the analysis, but there was also an increase in the concentration of the three nonaBDE congeners, from 5,743 to 8,950 µg/kg dry weight. It should be noted that the mean measured decaBDE concentration did not change significantly over this period (although the variability was high).

Table 8**:** Formation of lower PBDE congeners from the anaerobic degradation of decaBDE (nominal 500 mg/kg treatment, composite sample) (Schaefer & Flaggs, 2001b)

Congeners	**Molecular weight (g/mol)**	**Concentration in experimental samples (ng/kg dw)[a]**		**Percentage increase in number of moles**
		Day 0	**Week 32**	
2,2′,4,4′-TetraBDE	485.8	1,600	4,080	155
2,2′,4,5′-TetraBDE		129	804	523
2,3′,4,4′-TetraBDE		<41.5	277	>567
2,3′,4′,6-TetraBDE		<32.9	139	>322
2,4,4′,6-TetraBDE		<27.1	33.1	22
3,3′4,4′-TetraBDE		<23.1	<11.3	-
2,2′,3,4,4′-PentaBDE	564.7	<154	361	>134
2,2′,4,4′,5-PentaBDE		2,460	5,130	108
2,2′,4,4′,6-PentaBDE		363	992	173
2,3,3′,4,4′-PentaBDE		<235	459	95
2,3,4,5,6-PentaBDE		<242	1,120	>362
2,3′,4,4′,6-PentaBDE		<122	235	>93
3,3′,4,4′,5-PentaBDE		<93.6	<87.8	-
2,2′,3,4,4′,5′-HexaBDE	643.6	1,620	2,080	28
2,2′,3,4,4′,6′-HexaBDE		345	1,710	396
2,2′,4,4′,5,5′-HexaBDE		11,100	15,900	43
2,2′,4,4′,5,6′-HexaBDE		1,670	2,790	67
2,2′,4,4′,6,6′-HexaBDE		<106	<51.1	-
2,2′,3,4,4′,5,6-HeptaBDE	722.5	2,020	11,100	450
2,2′,3,4,4′,5′,6-HeptaBDE		47,000	67,700	44
2,3,3′,4,4′,5,6-HeptaBDE[c]		7,530	31,900	323
SUM[b]		**~76,380**	**~146,900**	**-**

Note: a – Standard deviation data are not available in the study report.
b – 'Less than' values are taken as half the reported value for this calculation.

c – No octaBDE congeners were included in the analysis.

b) In the 5 mg/kg treatment, the levels measured in week 32 appeared to be broadly comparable with the levels measured on day 0 or in the laboratory blanks for all congeners, although this is complicated by significant analytical variability. For example, for 2,2′,4,5′-tetraBDE the starting concentration was 95.6 ng/kg dw whilst the final concentration was 1,020 ng/kg dw in the composite sample but 121 ng/kg dw from the mean of duplicate analyses of a single sample. Similarly, for 2,2′,4,4′,5-pentaBDE the initial concentration was 1,910 ng/kg dw whilst the

final concentration was 6,760 ng/kg dw (composite sample) or 1,295 ng/kg dw (mean of duplicate analyses of the same sample).

The registration dossiers conclude that decaBDE was neither mineralised nor biotransformed under anaerobic conditions in a flooded sediment over a 32-week period. However, this experiment has to be interpreted with some caution for the following reasons:

- Samples were randomly selected and so the test vessel replicates analyzed at the end of the test were not necessarily the same as those at the start. This is important because vessels containing a greater amount of gravel/stones had proportionately less sediment and were a source of variability between replicates.

- Owing to the small sample size and high variability in analytical results, it was not possible to determine whether the observed differences were statistically significant (e.g. it is possible that they might have been due to the random errors inherent in the experimental methodology).

- Only 3 of 24 heptaBDE, 5 of 42 hexaBDE, 7 of 46 pentaBDE and 6 of 42 tetraBDE congeners were quantified due to a lack of available analytical standards. Whilst not all congeners may be relevant members of the degradation pathway, only a partial picture might have been provided.

- Degradation rates may be governed by the availability of the substance to micro-organisms, and the influence of different loading levels on this is unknown. The highest treatment level is an order of magnitude greater than sediment concentrations typically encountered in the environment. For example, the highest reported sediment concentration cited in the risk assessment reports is 12.5 mg/kg dry weight for a Spanish river close to sources of release (Eljarrat et al., 2007). However, it is possible that levels could be higher at other locations, since the number of sampling sites is limited.

- This test used a sediment with a low organic carbon content (i.e. availability might have been relatively high compared to other sediments).

Overall, the observations from this test provide, at most, equivocal evidence of transformation.

5) KEMI (1994) and de Wit (2000) reported that no degradation/transformation of decaBDE was seen after four months' incubation in sediment samples under anaerobic conditions. The inoculum used was an enrichment culture from a PBDE-contaminated sediment. The incubation of one of the anaerobic cultures was extended to two years, but no degradation was seen over this time period either. No further details of this test were reported (e.g. analytical detection limits and variability, whether attempts were made to look for low levels of degradation products, etc.), so the reliability and relevance of this study cannot be determined.

6) Tokarz et al. (2008) showed that decaBDE degraded over a 3.5 year period in sediments held in the dark at 22°C in a laboratory microcosm experiment. (This study was not summarised in previous EU risk assessment reports.) A degradation experiment was also performed using a co-solvent enhanced "biomimetic" system with titanium citrate and vitamin B_{12}. However, since this is not particularly environmentally relevant, only the details of the sediment microcosm part of the study are summarized in this dossier.

Sediment microcosms were made using a natural loam sediment (which contained no detectable PBDEs) with a pH of 6.3 and an organic carbon content of 16.4%. The test substance used in the study contained small amounts of nonaBDE (2.0% BDE-206, 1.9% BDE-207 and 0.9% BDE-208 on a mole fraction basis). DecaBDE (3.5 mg) was firstly added to 10 g of air-dried, sieved (2 mm) sediment as a solution in toluene and the toluene allowed to evaporate. The spiked dried sediment was then mixed into 70 g of sieved wet sediment (water content approximately 50%) giving a final decaBDE concentration of 5.0 mg/kg [Note: the paper indicates that the final concentration was 5.0 mg/kg but this does not appear to be correct as a total of 3.5 mg of decaBDE was added to 80 g of sediment – the initial concentration would therefore appear to be approximately 3.5/0.080 = 44 mg/kg]. The spiked sediment was added to 125 ml serum bottles containing 50 ml of phosphate buffer and the sediments were fed with 50 µl of methanol and 25 mg of dextrose in order to provide an organic electron donor and to ensure anaerobic conditions (the combination of dextrose and methanol promotes the rapid onset of anaerobic conditions without the need for adding exogenous reducing chemicals). The microcosms were sealed, shaken, and then incubated in the dark at 22°C. Three replicate microcosms appear to have been prepared (although this is not altogether clear from the paper). Control microcosms were prepared in a similar manner and were autoclaved three times prior to use. In addition, a second set of sediment microcosms that had been constructed three years earlier were also used in the study (the concentration of decaBDE in these microcosms was reported to be 0.3 mg/kg).

The methane gas produced by the sediment microcosms was measured by inserting a glass-barrelled syringe through the stopper. At intervals during the test, sub-samples of the sediment were collected and analysed for the presence of PBDEs.

DecaBDE was found to degrade very slowly in the sediment microcosms. For example, it was found that only a very slight decrease in the amount of decaBDE was evident after 10 months' incubation, with a concurrent increase in all three nonaBDEs. However, more extensive degradation was evident in the older microcosms after 3.5 years of incubation. The amount of decaBDE present at the end of the study period was around 55% of that initially added (0.3 mg/kg) in one replicate, around 80% of that initially added in the two other replicates and around 85% of that initially added in the abiotic control (values read from a graph). In the replicate showing the greatest level of loss, a statistically significant increase in the mole fraction of all three nonaBDEs was evident, along with the appearance of a number of hepta- and octaBDEs, plus a small amount of hexaBDEs (e.g. BDE-128 and BDE-138). The mole fraction of the hexa- to heptaBDE congeners was roughly 1% by the end of the experiment in this replicate (read from a graph)[16]. The concentrations are low, and the influence of analytical variability on these results is unknown, but they were not detected in the control.

Only one of the three replicates appeared to show significant degradation, and there is little information about how any of the microcosms were treated

[16] The mole fraction of the five octaBDE congeners detected was roughly 9% (read from a graph) for this replicate, compared to below about 1% in the control (which only contained two of the congeners). The same octaBDE congeners were also observed in the other two replicates, but at lower levels.

during the 3.5 year period (e.g. whether feeding with methanol and dextrose was carried out at intervals over this time period, etc.), so their individual viability is unclear. The half-life of decaBDE in this test system was estimated to be between 6 and 50 years, with an average of around 14 years. The study was carried out at 22°C and so at more environmentally-relevant temperatures the degradation would be expected to be slower.

The source of the sediment is not given in the paper, but if it was the same as used for a more recent sediment microcosm reported in the same paper, it might have had a high organic carbon content (16.4% organic carbon, compared to the 5% that is assumed in the REACH Technical Guidance Document). In other words, adsorption is expected to have been higher and availability to micro-organisms lower than under 'typical' conditions. The results might therefore be unrepresentative for sediments with lower organic carbon contents

Given the uncertainties, this study provides equivocal evidence for transformation. The main implication is that there may be a long time lag between disappearance of decaBDE and the subsequent formation of the lower PBDE congeners in some sediments. In other words, the degradation of decaBDE to lower PBDEs is possible, but is likely to be practically immeasurable in studies that take place over short timescales (i.e. days to weeks).

7) Parsons et al. (2004) [ABST], Skoczynska et al. (2005) [ABST] and Parsons et al, (2010) [ABST] investigated the potential for the anaerobic degradation of decaBDE in sediment samples collected from an area known to be contaminated with the substance (the Western Scheldt). It appears that more than one study was carried out, since the three summaries differ in some of their experimental details (e.g. the amount of sample), although they appear to have been broadly similar.

The samples were suspended in anaerobic medium containing acetate, lactate and pyruvate as electron donors. The suspensions were spiked with individual PBDE congeners (decaBDE, 14.04 µg/g) and incubated anaerobically at room temperature in the dark over ~210 days (or nine months). Autoclaved suspensions were incubated as sterile controls. The treated samples were extracted at intervals with hexane/acetone (or pentane/acetone) and analysed using gas chromatography-low resolution mass spectrometry (GC-LRMS).

A rapid decrease in the decaBDE concentration was observed during the first two months of one experiment, followed by a period showing no significant degradation. Removal of BDE-99 (2,2',4,4',5-pentaBDE) and BDE-183 (2,2',3,4,4',5',6-heptaBDE) was much slower. The GC/MS chromatograms of samples taken during the course of the experiment with decaBDE showed that new peaks appeared with retention times slightly shorter than that of the parent substance, which were identified as the three nonaBDE congeners (by comparison of their retention times and mass spectra with authentic samples). The amounts of these debromination products were not reported. No octaBDE congeners were detected in either the sampled sediment or in the incubations with decaBDE.

Important experimental details are missing (e.g. there are no details about the sediment spiking technique, or the background levels of decaBDE and other PBDEs in the sediments used). Some unusual observations during one test are also not clearly explained. For example, the measured decaBDE

concentration at the start of this test appeared to be only around 25% of the nominally added amount. Decreases in the sediment concentration of decaBDE were observed at a similar level in the controls as the treatments. The control sediment emitted methane after addition of lactic, pyruvic and acetic acids, which suggests that sterilization was incomplete.

In view of the limited details available for this study series, and the fact that they appear to show some serious shortcomings in the test, the results are not considered further in this report.

8) Rheinstein (2006) and Rheinstein et al. (2006) [ABST] investigated decaBDE degradation over six months in anaerobic sediments collected from Hamilton Harbour, situated at the western edge of Lake Ontario, Canada (43° 17' N, 79° 50' W). [This study has not been previously evaluated and is not included in the registration dossiers.] The sediments were fine grained with a water content of ~80% and an organic carbon content of ~8% dw. Five sediment cores were collected from an area near the centre of the harbour at a depth of around 24-25 metres in May and August 2005. Each core was sub-sampled with three Plexiglas vertical cores measuring 30 cm in length and 6 cm in diameter. The harbour has very low oxygen levels in the bottom layer of water, so immediately following sub-sampling, the vertical cores were capped and taped for transport to the laboratory. The cores were placed in an anaerobic chamber (supplied with an atmosphere of argon/hydrogen in the ratio 95:5), and the overlying water and aerobic sediment (indicated by a lighter colour) were discarded. Four microcosm treatments were prepared using the top 5 cm of anaerobic sediment from nine core sub-samples (giving 30 g of wet sample for each replicate): an untreated control, sterilized control, 100 ng decaBDE spike (low dose), and 1666.67 ng (1.74 nmol) decaBDE spike (high dose), with three test vessels per treatment:

   - The sterilized control was prepared by autoclaving the sediment sample in a sterilized amber glass jar (to minimise photodegradation) at 120 °C and 20 pounds per square inch [138 kPa] for 20 minutes, followed by cooling in the anaerobic chamber, addition of 0.5 g of glucose in 2 ml of deionised water (as a nutritional source) and homogenization by hand mixing with a clean plastic spoon. The jars were then capped and sealed with parafilm.

   - The high dose treatment was prepared as follows. A ^{12}C-decaBDE standard from Wellington Laboratories (Guelph, Ontario) containing 50 ng/µl decaBDE (purity not stated) in toluene was diluted with 120 ml of toluene to produce a decaBDE concentration of 0.2 ng/µl. This solution was mixed using a stainless steel spatula with 120 g of a dried, ground sediment that had previously been collected from Lake Ontario. The sediment was spread onto a tray and allowed to dry for 48 hours in a fume cupboard, before 5 g of the treated sediment was added to an amber glass jar and rehydrated with 4 ml of water. The jars were taken back to the anaerobic chamber and 30 g of Hamilton Harbour sediment was added to each, followed by glucose solution as described above. The mixing time was not reported, although since it was by hand, it was probably in the order of minutes. It was not stated whether the jars were sealed or left open.

Three test vessels were incubated over a 27-week (189-day) period. The temperature was not reported. It is presumed (but not stated) that the incubations were performed in the anaerobic chamber. Samples were collected for analysis eight times (in weeks 0, 2, 5, 8, 12, 17, 22 and 27). At each sampling

point, samples (n = 3) were removed from each treatment and freeze dried at -50 °C for 3-4 days, prior to grinding. The resulting samples (5 g) were then extracted using an Accelerated Solvent Extraction system and cleaned up (including a silver nitrate treatment to remove sulfur) for PBDE analysis using high resolution GC/MS. Since decaBDE levels in the untreated control and low dose treatments could not be distinguished from those in laboratory blanks, only the sterilized control and high dose treatments were carried through to completion. Sediments for both of these treatments were in fact collected on the same day in August.

Four phases were apparent in the high dose treatment, relating to changes in the decaBDE level (reported as the mean of three replicates):

- Day 0 to 14: The nominal decaBDE concentration in sediment resulting from the spiking procedure was 327.7 ng/g dw. At the start of the test, only 125.9 ng/g was detected (i.e. 38% of nominal, ignoring historical contamination). The measured concentration rose to 272.1 ng/g dw by day 14 (i.e. 83% of nominal). An average 10% loss of organic carbon also occurred over this time period, possibly caused by stimulation of microbial metabolism prompted by the glucose addition. This change might be linked to the apparent increase in decaBDE 'availability'.

- Day 14 to 35: The decaBDE concentration dropped by 52.6% to 128.5 ng/g dw by day 35, although this change was said to be not statistically significant.

- Day 35 to 119: The decaBDE concentration rose to 232.6 ng/g dw by day 119 (an increase of 81% compared to the level on day 35), but again, this was said not statistically significant. There was a further decrease in organic carbon of about 4.6%.

- Day 119 to 189: The decaBDE concentration decreased by 47.4% to 122.1 ng/g dw by the end of the study.

- No statistically significant decrease in decaBDE concentration was observed when the whole 189-day period is considered.

The results for the sterilized control treatment are not discussed, but they are presented graphically. They appear to show a consistent decaBDE level of roughly 25 ng/g dw throughout the incubation. The report states that the sampled sediments had a background decaBDE concentration of 15.4 ng/g dw, although it is not clear which samples were used to derive this value.

Variations in concentration of three nonaBDEs (BDE-206, -207 and -208) were apparent, and the mean concentrations at the end of the 189-day period were all lower than at day 14 (but higher than day 0). The differences were small (in the region of 1-3 ng/g) and the changes were not statistically significant between days 14 and 189 (there was high variation between samples at some sampling points). This change might therefore simply indicate a time lag in reaching equilibrium following spiking (the purity of the test substance is not stated, so these congeners might have been present in the spike). The possibility that these congeners were already present due to historical contamination could not be discounted either, based on the analytical method that was used.

The wide variation in decaBDE concentrations over the course of the experiment, and the inconclusive data on nonaBDE, makes it impossible to determine whether any degradation was occurring. Fluctuations were considered to be due to a

number of possible factors, including poor homogenization during the sediment spiking technique (resulting in heterogeneous dispersion of the test substance, and possibly historical contamination, in the sediment), insufficient time allowed to reach equilibrium, the influence of mineralogy (including sulfur) and metal contamination (the sampled sediments had elevated levels of several metals, and also sulfur at a concentration of 4.1 mg/g dw, which is around 140 times higher than Lake Ontario levels), and/or variations in organic carbon content or microbial activity. Limitations of the extraction technique was also a possible factor. The sample size at each sampling point is not altogether clear, and it appears that there was substantial reduction in the amount of remaining sediment as the test progressed towards completion. Overall, this is not a reliable study, and cannot be used as part of any weight of evidence analysis.

DNA fingerprinting did not reveal the presence of three known microbial polychlorobiphenyl (PCB) or PBDE degraders (*Dehalococcoides ethanogenes*, *Desulfitobacterium dehalogenans* and *Sulfospirillum multivorans*), although a substantial amount of bacterial DNA was present.

9) One government-funded laboratory has investigated decaBDE's behaviour using mesocosm test systems (Feibicke et al., 2009 [ABST])[17]. Full study details are not publicly available, but some information has been presented in two conference posters and further information has been provided through correspondence with the study authors for the purposes of this evaluation (M. Feibicke, personal communication, June 2012).

The study was initiated in July 2008 using artificial indoor ponds were equipped with transparent covers to avoid input from dust. Two ponds containing equal amounts of sediment were dosed with an initial decaBDE concentration of 100 ng/L (together with two other brominated flame retardants). The test substance solution was prepared in tetrahydrofuran/toluene/iso-propanol (in the volume ratio 30/30/140), and was applied with a low pressure water jet sprayed through a flat nozzle directly onto the water surface. Lithium bromide and uranine (CAS no. 518-47-8) were also added as tracers to check homogeneity and calibration of pond water volume. An untreated control pond with sediment was also prepared. The ponds had a water volume of 22-25 m^3 (i.e. up to 25,000 litres), with dimensions of 6.9 x 3.3 x 1.0-1.1 metres (length, width and height, respectively). The total sediment depth was 0.8 m (although only the surface layer (10 – 20 cm depth) was considered relevant for water-sediment interactions). The pond walls were made of gel-coated fibre-reinforced composite material, and gel coated fibre tiles were placed at a depth of 10 cm to allow a biofilm to develop. The sediment was a natural fine particulate (sandy) lake sediment. The top layer was a mixture of sand and fine particulate lake sediment. Organic compounds were most prevalent at the surface and decreased with sediment depth, and iron hydroxide was added to condition the sediment before the study began. The sediment surface area was 22.77 m^2, so the water volume-to-sediment area ratio was approximately 1:1.

Water was circulated in each pond by bubbling cleaned compressed air. The ponds were covered with plastic covers (free from brominated flame retardants) to prevent contamination with dust, and macrophytes and invertebrates were introduced (these had been raised in indoor culture ponds): macrophytes (*Potamogeton nodosus* and *Myriophyllum spicatum*) were allocated to specific positions (five specimens of each per pond), and snails (*Lymnaea stagnalis*) and

[17] Meinecke et al., 2007 [ABST] presents the results of a pilot study from 2006, which was used to check the application device, sampling methods, etc., in preparation for the more detailed study.

water slaters were introduced in equal numbers. Phyto- and zooplankton were introduced by field inocula from a mesotrophic lake. The ponds were illuminated with an artificial light source, i.e. four HQI lamps (two 2000 W and two 400 W) and two daylight fluorescent tubes (58 W) per pond. The pond covers were opaque to UV radiation, so special UV transparent covers were mounted in the central part of the ponds to allow UV-A penetration to the water surface by the two central HQI-lamps (2000W), which were mounted above the cover. Two additional UV-A and two UV-B tubes (38 W and 40 W, respectively) were mounted under the dust covers at one end of the ponds. Light intensity was assessed spectrophometrically some months after the end of the study by use of other empty ponds which had been equipped identically. The irradiance of the ponds at visible light wavelengths (400 – 800 nm) was in the range 1,378-1,788 μW/cm^2 (for ponds without additional UV bulbs) and 1,452-1,596 μW/cm^2 (for ponds with additional UV bulbs). Similarly, ponds without UV bulbs received UV-A wavelengths (320-400 nm) at an intensity of 31-52 μW/cm^2, whereas those with UV bulbs received UV-A wavelengths and UV-B wavelengths (284-320 nm) in the ranges 126-171 and 33-47 μW/cm^2, respectively. The ponds did not receive equal intensities of all wavelengths across the water surface. The influence of this on the results is unknown.

The treatments lasted 191 days. The measured lithium tracer concentrations indicated a homogeneous distribution of the applied dose one hour after application. Samples were collected on several occasions up to about day 40, and then on days 70, 120 and 191 (values read from a graph). At each sampling point, mixed duplicate water samples (from various depths) were directly collected in submerged glass bottles. Duplicate sediment cores were sampled using a modified Berggren sampler on each sampling day (only the top 2.5 cm layer was used for further analysis due to limited analytical capacity, which might have led to an under-estimate of actual concentrations). Biofilm was sampled by scratching the surface of the exposed bags with a stainless steel scraper, and other biota were sampled manually (macrophytes, filamentous algae and snails). Samples were prepared for analysis using liquid/liquid extraction (water) or Soxhlet extraction (biofilm) with toluene, followed by clean-up with a multi-layer column containing neutral and acidified silica gel. Sediment samples were extracted using pressurized liquid extraction with toluene and in-situ elemental sulfur removal using activated copper granules, followed by clean-up with gel permeation chromatography and a multi-layer column containing neutral and acidified silica gel. Following extract concentration, the samples were analysed by short column gas chromatography-electron capture negative ionization mass spectrometry in SIM mode using internal standardization with 4',6-difluoro-2,2',3,3',4,5,5',6'-octabromodiphenyl ether (99.4% purity) and ^{13}C-BDE-209.

Water temperatures were around 22-25 °C at the start of the experiment, falling to around 10 °C by the end. Oxygen levels and redox profiles were not measured, but due to the presence of labile organic carbon and the dark/black colour of the sediment (indicating iron sulfide formation and sulfate reduction), it was considered likely that the bulk sediment was anaerobic (the top few millimeters could have contained oxygen via diffusion from the water). The analytical recovery rate for decaBDE was 90 – 97%. Water concentrations declined rapidly, with a DT_{50} between 3.9 and 5.5 days (following simple first order kinetics).

No detailed information is available about the decaBDE (or transformation product) concentrations at the end of the study. DecaBDE levels in the control pond were low for the whole study period (read from a graph). In the absence of a clear decreasing trend, it was concluded that decaBDE showed "no relevant degradation" in sediment over 191 days. In addition, preliminary results were said to indicate that no debrominated degradation products were formed either in

water or sediment. It is not yet known whether the mass balance was close to 100% (it is known for one of the other flame retardants used in the study that around 40% of the applied amount was found in the sediment, with about 20% of the nominal dosed mass remaining on the pond wall after 346 days).

Although the test was designed to deliver a homogenous distribution of the applied substance, unavoidable patchiness would have developed during the course of the study due to different processes (e.g. local air bubbling affecting particle transport and sedimentation, small scale growth of submerged macrophytes influencing water turbulence and sedimentation, induced local agglomeration of decaying plant material, heterogeneous mat-like growth of filamentous algae on the sediment and pond wall surfaces, etc.).

This seems to be an environmentally relevant simulation study. Due to the lack of information it is not yet possible to draw firm conclusions about the level of transformation. However, it would appear to show that decaBDE was persistent in this test system.

10) Sediment cores can be analysed to investigate whether PBDE congener profiles change with depth, which may provide some indication as to whether degradation is occurring. Several studies have been performed:

- de Boer et al. (2001) analysed eight sediment cores from various European locations. They found that decaBDE concentrations had increased in recent years, whereas a parallel increase in concentrations of PBDE congeners associated with the commercial pentaBDE product was not observed (except for one site at Drammenfjord, Norway). Since there was no indication of increasing levels of nona- or octaBDEs (on a qualitative rather than quantitative basis), the study authors concluded that it was unlikely that 'significant' amounts of lower PBDEs were being formed from decaBDE in sediment, unless degradation was occurring at an extremely slow rate. However, given the high concentrations that were detected, and the limited number of congeners examined (only one heptaBDE congener was included in the quantitative analysis, and nona- and octaBDE congeners were not quantified) this conclusion may be misleading. Zegers et al. (2000 [ABST] and 2003) reported some of the data provided by de Boer et al. (2001).

- Voorspoels et al. (2004a [ABST] and 2004b) investigated the correlation between the concentrations of decaBDE and the sum of specific tri- to heptaBDE congeners found in various sediments from the Belgian North Sea (six locations), the Western Scheldt estuary (9 locations) and several freshwater tributaries (14 locations) of the river Scheldt. The samples were collected to a depth of 20-25 cm. The decaBDE concentrations found were in the range 1.1-24 µg/kg dry weight in samples from the Belgian North Sea (detected in 83% of the samples), 1.5-1,200 µg/kg dry weight in samples from the Scheldt estuary (detected in all nine samples) and <0.1-320 µg/kg in samples from the tributaries of the river Scheldt (detected in 86% of the samples). DecaBDE was the most abundant congener found in the samples accounting for around 95% of the total PBDE concentration in the Scheldt estuary samples and 52-99% of the freshwater tributary samples (it was also the predominant congener found in the Belgian North Sea samples). A statistically significant (p=0.05) positive correlation between the concentration of decaBDE and that of the lower PBDE congeners was found for the samples from marine locations (Belgian North Sea and Scheldt estuary)

whereas no correlation was evident in the samples from the freshwater locations.

- Moon et al. (2007a [ABST] and 2007b) collected surface (0–4 cm depth) sediment samples at 111 locations within three industrialised bays in South Korea (Ulsan Bay, Busan Bay and Jinhae Bay). The samples were collected between February 2003 and March 2004. DecaBDE was found to be present in all samples at a concentration between 2.0 and 2,248 µg/kg dry weight and was the predominant PBDE in the samples. There was also evidence of pollution by the commercial octaBDE product at some locations, although it appears that no nona- or octaBDEs were included in the analysis. No correlation was found between the levels of decaBDE present and the levels of six tri- to hexaBDEs present in the samples. (This study was not summarised in previous EU risk assessment reports.)

- Kohler et al. (2008) analysed dated sections of a sediment core collected from the deepest part (31 m) of Greigensee, a small urban lake close to Zürich, Switzerland, in April 2003 (taking precautions during the analysis to prevent photodegradation):

  - The level of decaBDE was found to be 7.2 µg/kg dry weight in the layer corresponding to 2001, decreasing to 6.7, 4.1, 1.9 and 1.1 µg/kg dry weight in the layers corresponding to 1995, 1989, 1982 and 1974 respectively.
  - The level of nonaBDEs (the sum of BDE-206, BDE-207 and BDE-208) was found to be 0.26 µg/kg dry weight in 2001, decreasing to 0.16, 0.12 and 0.03 µg/kg dry weight in the layers corresponding to 1995, 1989 and 1974 respectively (the 1982 layer was not analysed for nonaBDE). The congener pattern was broadly similar in all years (BDE-206 ≈ BDE-207 > BDE-208), implying that there was no long-term transformation of these congeners in the sediment. However, the authors stated that this distribution does not reflect that seen in commercial PBDE products, which might reflect differences in sources, partitioning behaviour, or degradation of decaBDE.
  - The octaBDE congener profile was also relatively consistent, with BDE-197/204 ≈ BDE-193/203 ≈ BDE-196/200 ≈ BDE-201 > BDE-202 > BDE-205 ≈ BDE-194, suggesting that no major transformation processes were occurring in the sediment. Again the congener profile did not correspond with the profiles found in commercial PBDE products.
  - The detection of BDE-202 might be suggestive of transformation, since it has been detected in anaerobic degradation studies (e.g. Gerecke et al., 2005) and was not found in significant amounts in commercial PBDE products by the authors[18]. However, the supporting information indicates that BDE-202 was also present in a sample of "clean" historical sediment (corresponding to the year 1848) that had been spiked with a commercial decaBDE product.

[18] The actual commercial products analysed included two octaBDE products from Great Lakes (DE-79 and another unknown product) and three decaBDE products (Bromkal 82-ODE, an unknown product from Great Lakes and a technical product of 98% purity from Aldrich). No BDE-202 was detectable in the three decaBDE products or one of the octaBDE products but a trace amount of BDE-202 was found in the other octaBDE product. The BDE-202 content of other products which are currently supplied, or have been supplied in the past, is unknown.

(This study was not summarised in previous EU risk assessment reports.)

- In contrast, Bradley et al. (2011) analyzed 23 tri- to heptaBDEs (and 12 methoxy-PBDEs) in dated sediment cores collected from two inland lakes (White Lake and Muskegon Lake) in Michigan, USA. A different temporal trend for BDE-183 (a heptaBDE) was found compared to the other PBDEs, which the authors suggested is consistent with debromination of higher molecular weight PBDEs during sedimentation and aging. (This study was not summarised in previous EU risk assessment reports.)

11) Leslie et al. (2008) and Leslie & de Boer (2010)[19] measured 3,3',4,4',5-pentaBDE (BDE-126) concentrations in estuarine sediment samples collected as part of an Industry-funded ten-year monitoring programme in response to Commission Regulation (EC) No. 565/2006[20]. This congener was specifically chosen as a representative marker for abiotic decaBDE degradation and had not been detected in commercial PBDE products up to the point of its inclusion in the monitoring suite in 2006. BDE-126 was not detectable (the detection limit was <0.008 to <0.05 µg/kg wet weight) in twenty-four sediment samples collected during 2006. In contrast, this congener was found to be present above the limit of detection (0.03 µg/kg dry weight) in five of eight sediment samples collected during 2007 (from the Western Scheldt, Elbe, Eems and Seine estuaries). Most of the concentrations could not be accurately quantified, but the approximate concentration was in the range 0.03 to 0.29 µg/kg dry weight. (Leslie et al., 2007). BDE-126 was not detected in eight sediment samples collected in 2009, at a detection limit of 0.04 – 0.09 µg/kg dry weight.

The levels are low, but if the BDE-126 is indeed derived from decaBDE, higher molecular weight PBDE congeners would also be present as intermediate degradation products, and it is likely that other pentaBDEs would also be formed through competing reactions Comprehensive PBDE analysis is not being performed for this study.

This finding provides equivocal evidence for decaBDE transformation, since detection in samples collected in 2007 but not 2006 or 2009 is hard to explain, although this may be due to the very low levels. It is possible that this congener was a contaminant of commercial PBDE products used in the past, or is a breakdown product of commercial penta- or octaBDE products that are still being emitted from treated articles. (These studies have not been discussed in an EU context previously.)

Discussion

A wide range of information is available on transformation of decaBDE in sediment. Qiu et al. (2011) have demonstrated that sediment-dwelling micro-organisms are capable of carrying out transformation reactions to form at least hexaBDEs under laboratory conditions over a three-month period. Similar findings have been reported by other laboratories (e.g. Deng et al., 2011). Preliminary results of Canadian-government funded studies provide evidence of the formation of small amounts of nona- and octaBDEs over 30 days in lake sediment under relevant environmental conditions. The findings still require confirmation with

[19] Sediment is being sampled once every two years. The report for 2008 (Leslie et al., 2009) therefore does not present any data for this matrix.

[20] O.J. No L 99, 07/04/2006 p. 003 - 005.

more specific analytical techniques, and the limited duration of the studies means that the results cannot be extrapolated to longer timescales.

Previous studies have provided only limited evidence of this process. For example, two laboratory studies (Schaefer & Flaggs, 2001a & 2001b, and Tokarz et al., 2008) suggest an increased formation of hexa- to heptaBDE congeners over time frames of several months to three years, but methodological and analytical limitations mean that the reliability and representivity of these findings can not be established with any certainty. On the other hand, one study (de Wit, 2000) apparently found no evidence of degradation in sediment over four months to two years, although the reliability and relevance of this finding cannot be assessed due to lack of detail. Two further laboratory studies (Rheinstein (2006)/Rheinstein et al. (2006) [ABST] and Feibicke et al., 2009 [ABST]) do not provide any evidence of transformation in sediments, over timescales up to 190 days. Methodological limitations in the first study and lack of detailed information for the second mean that the results need to be considered with caution.

The available sediment core studies provide equivocal evidence of the importance of transformation in sediments. Kohler et al (2008) found no clear evidence of long-term transformation of decaBDE to nona- and octaBDE congeners in sediment cores, although the nonaBDE congener profile did not match known commercial products and a possible marker for transformation (an octaBDE congener) was detected (although this could have been a laboratory contaminant). Another study suggested that the temporal trend for a heptaBDE congener was an indication of transformation (Bradley et al., 2011). One study (Voorspoels et al., 2004a [ABST] and 2004b) found a statistically significant positive correlation between the concentrations of decaBDE and the sum of specific tri- to heptaBDE congeners in various marine sediments, but not in freshwater sediments. Although other studies (de Boer et al., 2001; and Moon et al., 2007a [ABST] and 2007b) do not provide evidence of transformation (or suggest that transformation is very slow), they did not investigate hepta-, octa- or nonaBDEs properly, and so they are of limited relevance. There are several confounding factors in the interpretation of such studies. These include changes in product purity or emission pattern with time[21], emissions of lower congeners from other PBDE products which could mask the pattern of any degradation from decaBDE, and lack of comprehensive congener analysis (as well as possible false positives and negatives in earlier studies). In addition, the cores will only reflect conditions at the locations they were taken from, and so their representivity of the wider environment is unclear.

A possible marker for transformation to at least pentaBDEs has been found in a small number of sediment samples collected during 2007 but not 2006 or 2009 (Leslie et al., 2008; Leslie and de Boer, 2010), although this is only equivocal evidence.

Several factors could affect the degradation rate in any particular sediment, and it is not yet possible to establish the relative importance of these:

- It is possible that degradation kinetics vary with sediment loading, related to the concentration of the substance required to activate microbial metabolism. Transformation is most readily apparent at higher sediment loadings,

[21] For example, BSEF (2009) has indicated that the purity of the commercial decaBDE supplied by the three major EU importers has been higher than 97% for over ten years, but the congener profile of historical production, as well as current production by other companies, has not been so well characterised.

although this may be due more to analytical detection limits than variation in rates.

- Microbial biomass, presence of dehalogenating species, and the degree of adaptation of the micro-organisms to PBDE (or other halogenated substance) exposure.
- The chemical composition of the sediment can influence degradation rates due to microbial toxicity (e.g. metals) or the availability of electron donors and acceptors (halorespiration may be inhibited by the presence of more available alternate electron acceptors such as sulfate and nitrate, e.g. Hartkamp-Commandeur et al., 1996; Haggblom et al., 2000).
- The availability of the substance to micro-organisms (or for other processes) might be influenced by adsorption to/desorption from the matrix (related to mineralogy (which might also affect reactivity, e.g. Ahn et al, 2006a), microporous black carbon and organic carbon content), as well as the way that the substance is introduced to the system.[22]

3.1.2.3 Biodegradation in sewage sludge

The registration dossiers do not provide any information on this end point.

Four studies are available. [A fifth study by Olsman et al. (2007) that used digested household waste from semi-continuous laboratory scale mesophilic and thermophilic digesters provides no information on decaBDE concentrations and so is not considered further in this dossier.]

1) The degradation of PBDEs including decaBDE in sewage sludge has been investigated by Stiborová et al. (2008a) [ABST][23]. (This study was not summarised in previous EU risk assessment reports.) Sludge was collected from two wastewater treatment plants from the Czech Republic. PBDEs initially present in the sediment included BDE-28, -47, -49, -66, -85, -99, -100, -154, -183)[24] at a total concentration of 920.9 and 220.4 µg/kg dry weight in the two sludge samples, along with decaBDE at a concentration of 685.3 and 1,402.6 µg/kg dry weight respectively. The samples from each plant were pooled in separate jars and stored on ice during transport, and then at 4°C for up to four weeks before use.

   Degradation was investigated under both aerobic and anaerobic conditions for both sets of sludge samples:

   - For the aerobic experiment, slurries were prepared by mixing 15 g of wet sewage sludge with 35 ml of mineral medium. Three conditions were tested, using three parallel flasks for each. The first series of flasks contained the sewage sludge slurry alone. The second series contained the sewage sludge slurry amended with yeast extract (50 mg/l). The third

[22] Klosterhaus and Baker (2010) observed that decaBDE present in field sediments had a substantially lower bioavailability to polychaete worms than that in freshly spiked samples over 28 days' exposure.

[23] The same experiment is also summarised in Stiborová et al. (2008b) [ABST].

[24] These were the congeners analysed for. It is possible that other congeners may have been present that were not detected by the analytical method used.

series contained the sewage sludge amended with both yeast extract (50 mg/l) and 4-bromobiphenyl[25] (0.6 mg/l) as a 'primer'. Control flasks were prepared by heat-sterilisation of the sludge. The flasks were then incubated in the dark at 28°C with constant shaking (150 rpm) for three months.

- The anaerobic experiments were carried out by suspending the sewage sludge samples in a mineral medium (40:60 ratio sludge:medium; total volume 50 ml) in capped serum bottles. Starch (20 mg) and yeast extract (50 mg) were also added to the test system. A second series of experiments was carried out with the addition of starch, yeast extract and 4-bromobiphenyl (amount not given). Sterile control bottles were also prepared in the same manner as for the aerobic tests. The bottles were incubated in the dark at 28°C with constant shaking (150 rpm) for six months. Shortly after incubation had started, gas production was noted in the bottles indicating that methanogenic conditions had been attained.

For chemical analysis, sludge samples were dried and extracted with dichloromethane in a Soxhlet apparatus, followed by gel permeation chromatography and quantification of the resulting solvent extracts by GC/MS-NCI. DecaBDE was extracted directly from the culture medium with isooctane.

At the end of the three-month period the amount of PBDEs present in the aerobic sludge slurries had decreased by around 30% (total of BDE-28 to BDE-183) and 20% (decaBDE). Two additional unidentified peaks were also observed in the sample chromatograms compared to the controls. Yeast extract and 4-bromobiphenyl had no significant effect on the loss seen.

Significant loss of decaBDE was also evident in the anaerobic experiments with the sewage sludge collected from one of the sites, but no data are reported. The concentration of decaBDE was found to remain constant in the experiments with the other sludge sample after six months. Loss of the lower PBDE congeners initially present was evident in both sludges (up to 50% in one sludge (which had the higher initial concentration) and up to 30% in the other). The loss was generally higher in the experiments where 4-bromodiphenyl had been added.

Few other details of this study are available. In particular no information is given on PBDE concentrations in the sterile controls at the end of the incubation period. Therefore the significance of the results cannot be assessed.

2) The degradation of decaBDE in sewage sludge under anaerobic conditions was studied by Gerecke et al. (2004 [ABST] and 2005). The experiments were carried out in 100 ml glass serum bottles each containing a 1 cm layer of glass beads. The bottles were spiked with stock solutions of decaBDE (98% purity; 10 nmole added to each bottle), alpha-hexachlorocyclohexane (used as a positive control; 10.5 nmole added), and five 'primers' (4-bromobenzoic acid, 2,6-dibromobiphenyl, tetrabromobisphenol-A, hexabromocyclododecane and decabromobiphenyl (around 9-11 nmol of each was added)). Two experiments were also carried out using either BDE-206 (2,2',3,3',4,4',5,5',6-nonaBDE) or BDE-207 (2,2',3,3',4,4',5,6,6'-nonaBDE).

[25] At one place in the paper this is referred to as 4-bromobiphenyl ether and so it is not clear exactly what was added.

The solvent was allowed to evaporate overnight (the solvent used was not stated) and then starch (20 mg), yeast (50 mg) and 20 ml of freshly collected digested sewage sludge (from a plant serving 45,000 people in Dübendorf, Switzerland) was added. The sludge used had a pH of 7.6, a solids content of 3% dry weight and was known to be contaminated with decaBDE (the concentration in the sludge was 58 nmole/l; the amounts of other PBDEs present in the sludge were not stated). The total amount of decaBDE in the system (spiked and from the sewage sludge) was 11.2 nmol/bottle. The bottles were then tightly capped and incubated in the dark at 37°C for up to 238 days. During the experiment (both incubation and analysis) exposure to light was kept as low as possible (for example windows and fume hoods were covered with a UV filter foil). Control experiments were carried out using heat-sterilized sludge.

Gas production was found to occur in all sample bottles indicating that methanogenic conditions were present. No gas production was found in the sterile controls.

The amount of decaBDE was found to decrease by around 30% (from the initial amount of 11.2 nmole to 7.9 nmole per bottle) after 238 days' incubation in the experiments with primers. The observed disappearance was found to be statistically significant at the 95% confidence level, and corresponded to a pseudo-first-order reaction rate constant of 1×10^{-3} day^{-1}. No significant degradation of decaBDE was seen in the sterile controls. The positive control (alpha-hexachlorohexane) was found to degrade with a rate constant of 0.4 d^{-1} indicating that the microbial community in the experiment was able to degrade halogenated compounds.

The study also investigated the amounts of several lower molecular weight PBDE congeners present at various times in the study. The decaBDE test substance contained traces of three nonaBDE congeners (BDE-206 ~2% on a molar basis; BDE-207 ~0.4% on a molar basis; and BDE-208 ~0.04% on a molar basis). OctaBDE congeners were not detected (the detection limit was 0.005 nmole/sample). During the incubations two nonaBDEs (BDE-207 and -208) and a number of octaBDEs were formed. The amount of BDE-208 present in the system was found to increase by more than ten times (from an amount below the limit of quantification to 0.15 nmole/bottle) and the amount of BDE-207 increased from 0.024 nmole/bottle to 0.16 nmole/bottle). Similarly the amount of octaBDEs increased from an amount below the limit of quantification to 0.21 nmole/bottle. In contrast, there was no statistically significant (95% confidence level) increase in the amount of BDE-206. It was suggested that this may have been due to either a very low formation rate from decaBDE, or a rapid degradation of BDE-206 itself. No nona- or octaBDEs were formed in the sterile controls. Overall, at least 0.5 nmole/bottle of transformation products were formed after 238 days' incubation, indicating that at least 4.5% of the decaBDE initially present in the system had degraded to lower PBDE congeners (nona- and octaBDEs).

The mass balance from the experiment showed that around 3 nmole/bottle of decaBDE degraded (from the initial amount of 11.2 nmol/bottle) but the amounts of octa- and nonaBDE congeners formed only accounted for around 0.5 nmole/bottle. There was no evidence of the formation of lower PBDE congeners (such as heptaBDEs) in the experiments. Explanations for this discrepancy could include formation of other unidentified transformation products, formation of bound (non-extractable) decaBDE residues, or imprecision in the analytical procedure used.

Experiments without primers showed that similar degradation products were formed but the rate of decaBDE degradation was approximately half of that found in the experiments with primers outlined above. The experiments with BDE-206 and BDE-207 both showed that the substances were degraded to octaBDEs but no rate constants for the reaction could be determined. Evidence was also presented that BDE-208 undergoes a similar degradation.

Overall this appears to be a reliable study. Around 30% of the decaBDE added to the test system with primers was transformed over 238 days at 37°C, and at least 4.5% was to nona- and octaBDEs. The results of this study appear to show that debromination of decaBDE proceeded most readily by loss of bromine from the *para*- (4-position) and *meta*-position (3- or 5-position) as shown by the formation of BDE-208 and BDE-207 (although degradation by loss of bromine from the *ortho*-position (leading to the formation of BDE-206) could not be entirely ruled out).

3) A further study on decaBDE using the same test system as above has been carried out by Gerecke et al. (2006). This paper reports the results of Gerecke et al. (2005) and the results of two new experiments where decaBDE was tested in the presence of a single primer (either 2,6-dibromophenol or 4-bromobenzoic acid). Formation of BDE-208 was found to be promoted using either of the primers. The estimated half-lives for degradation of decaBDE (based on two data points only) appear to be >700 days with 2,6-dibromobiphenyl and <1,400 days with 4-bromobenzoic acid.

4) Gerecke et al. (2005 and 2006) reported the results of a preliminary study to investigate if decaBDE degraded in a full-scale anaerobic digester. Grab samples of sewage sludge were taken from the mesophilic digester, including its inlet and outlet, at the same sewage treatment plant at Dübendorf that was used for the inoculum source in the experiments summarized above. These grab samples were analysed for the presence of PBDE congeners.

   The concentration of decaBDE was 76 nmol/l in the inlet sample, 58 nmol/l in the digester, and 49 nmol/l in the outlet. The concentrations of BDE-208 and BDE-207 were found to increase relative to that for BDE-206 between the inlet samples and the outlet samples, showing a similar pattern to that found in the laboratory experiment. The residence time in the reactor was 28 days.

   Although these data are suggestive that degradation of decaBDE was occurring in the digester, the authors cautioned that as the residence time in the reactor was 28 days, one set of grab samples does not provide unequivocal evidence that degradation of decaBDE was the source of these congeners in the samples. The temporal variability in the concentrations measured is not known. This study therefore provides equivocal evidence of transformation, but does not give any information about tetra- to heptaBDE congeners.

Some limited information is also provided by monitoring data. For example, Leslie et al. (2007 and 2008) and Leslie & de Boer (2010) analysed samples of sewage sludge collected from municipal WWTP in Europe during 2006, 2007 and 2009 for the Industry ten-year monitoring programme. (These data have not been summarised in previous EU risk assessment reports.) BDE-126 (a pentaBDE, assumed to be a marker for abiotic degradation) was not detectable (concentrations <0.01 µg/kg wet weight) in six sludge samples from 2006. BDE-126 was detected in both samples analysed in 2007, at a concentration of 0.10 µg/kg dry weight at one site and 0.11 to 0.15 µg/kg dry weight at the second site. In 2009, BDE-126 was detected in six out of twelve sludges that were screened, between the limit of detection and limit of quantification in the

approximate range 0.1 – 0.4 µg/kg dry weight (the other samples were below the detection limit of 0.1 – 0.3 µg/kg dry weight). If BDE-126 is indeed derived from decaBDE, higher molecular weight PBDE congeners would also be present as intermediate degradation products, and it is likely that other pentaBDEs would also be formed through competing reactions (comprehensive chemical analysis is not being performed). This finding is taken as equivocal evidence for transformation, since it cannot be entirely ruled out that this congener was a contaminant of commercial PBDE products used in the past[26]. In addition, it might not be associated with sewage treatment processes.

Discussion

Overall, these studies provide good evidence that decaBDE can be transformed to at least octaBDE congeners by sewage sludge micro-organisms over a period of about eight months. The amounts appear to be below 10% over this timescale, and the rate of reaction appears to depend on the presence of other substances. Whilst these findings do not suggest that tetra- to heptaBDE congeners would be formed in significant amounts during wastewater treatment processes (since sludge residence times are usually too short, at around 20 days), they do provide some supporting evidence that the reaction might occur over longer timescales in the environment under appropriate conditions.

3.1.2.4 Biodegradation in soil

The registration dossiers have two study summaries for this end point based on two references (Huang et al., 2010 and Sellström et al., 2005)[27]. The Sellström et al. (2005) study is indicated to be the key study, but it does not actually address biodegradation (see Section 3.1.1.2.3). The Huang et al. (2010) study is summarised below, together with additional information.

Huang et al. (2010) investigated the effect of plant growth on the degradation of decaBDE in soil under greenhouse conditions. (This study was not summarised in previous EU risk assessment reports.) The test substance purity was 99.5%, obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) (Zhang, 2010). No impurity information was provided, although from the initial soil concentrations (see below), it would appear that the main impurities were three nonaBDE congeners (BDE-207, -206, and -208). The soil was described as loamy (clay 23%, silt 35% and sand 32%) and had a pH of 7.32 and 3.11% organic matter[28]. The soil was air-dried, ground and passed through a 2-mm nylon sieve, before receiving mineral nutrients at rates of 100 mg phosphorus, 300 mg nitrogen, and 200 mg potassium per kilogram of soil as basal fertilizers. An aliquot of soil (1 kg, approximately 10% of the final amount) was spiked with a solution of decaBDE dissolved in 100 ml of a (1:10 v/v) mixture of toluene and acetone, mixed

[26] Leslie et al. (2008) also detected several PBDE congeners (e.g. BDE-48, BDE-99, BDE-100, BDE-153 and BDE-183) in sewage sludge samples collected in 2007 from Eindhoven and Kralingsveer in the Netherlands. These are known components of the commercial pentaBDE and octaBDE products. Their presence in sewage sludge implies a relatively recent emission to the WWTP. It could also imply some level of degradation of decaBDE during wastewater treatment, although this would appear unlikely based on the other data presented in this section.

[27] One of the study summaries also mentions a third reference (Banasik et al., 2009), although no further details are given, and in fact this does not address soil biodegradation.

[28] This is comparable with the generic assumptions in the REACH Technical Guidance (which adopts a weight fraction of organic matter in soil solids of 3.4%).

thoroughly, and placed under a fume hood for solvent evaporation for 12 hours. The spiked soil was then continuously tumbled with non-spiked soil for 2 hours at room temperature to ensure efficient mixing. The soil was then allowed to dry in the dark until the solvents had volatilized completely, shaken for 30 minutes every day, homogenized, and incubated in the dark for 4 weeks at room temperature. The measured concentration [with 95% confidence interval] of decaBDE in the soil after incubation was 4,960.1 [4,467 – 5,453] µg/kg. Three nonaBDE congeners (BDE-207, -206, and -208) were detected at concentrations of 23.5 [20.0 – 27.0], 38.2 [33.4 – 43.0], and 19.6 [17.2 – 22.0] µg/kg, respectively. Other PBDEs and hydroxylated-PBDEs were all below their analytical detection limit in the soil[29].

Italian ryegrass (*Lolium multiflorum*), alfalfa (*Medicago sativa cv. Chaoren*), pumpkin (*Cucurbita pepo ssp. Pepo cv. Lvjinli*), summer squash (*Cucurbita pepo ssp. Pepo cv. Cuiyu-2*), maize (*Zea mays cv. Nongda 108*), and radish (*Raphanus sativus cv. Dahongpao*) were used as the test plants. Each pot received 600 g of spiked soil. The upper 0.5-1.0 cm of each pot was covered with non-spiked soil (65 g) to establish a buffer layer to minimize PBDE evaporation and photolysis. Polyethylene bags were placed inside the pots to prevent contamination and water drainage. Ten pre-germinated seeds were sown in each pot and 3 days after emergence the seedlings were thinned to 8 for ryegrass, 5 for radish and alfalfa, 2 for maize, and 1 for pumpkin and summer squash, with the aim of obtaining an approximately equivalent amount of plant biomass per pot (the plant biomass ranged from 2.8-12.4 g per pot). Non-spiked soil with plant growth and spiked soil without plant growth were set up as the decaBDE-free blank and plant-free control, respectively. Four replicate pots of each treatment were prepared. Pots were kept in a controlled environment growth chamber for 60 days at a light intensity of 250 µmol/m^2/s provided by supplementary illumination with a photoperiod of 14 hours each day, at a 25/20°C day/night temperature regime, and a relative humidity of 70%. The pots were positioned randomly and re-randomized every two days. Distilled water was added as required to maintain moisture content at 60-70% of water holding capacity by regular weighing.

Root and shoot samples were carefully washed and then freeze-dried and ground. Soil and plant samples were submitted to Soxhlet extraction with a mixture of acetone and hexane (1:1). PCB-30 and PCB-209 were added as surrogate standards to the samples prior to extraction and BDE-77 was added to the final solutions as an internal standard. Analysis was performed using gas chromatography with microelectron-capture detection (GC-µECD). Whilst other techniques (e.g. GC/MS) might be preferable to identify PBDE congeners with certainty, the technique is considered to be sufficiently reliable in this case, given that they were not present at the start and other contaminants (like polychlorobiphenyls) should not have found their way into the matrix based on the precautions that were taken. Quality control included regular analyses of procedural blanks, blind duplicate samples, and random injection of solvent blanks and standards.[30]

[29] The detection limits were defined as three times the standard deviation of the levels found in the analytical (solvent) blanks (Zhang, 2010). Detection limits are given in the background information to the paper, and are often presented as a range for a number of congeners as a group.

[30] To determine potential degradation during the analytical method, anhydrous sodium sulfate was spiked with decaBDE and processed using the same extraction and analytical procedure. Only two nonaBDEs were detected at less than 0.02% of the decaBDE concentration, indicating that degradation to lower molecular weight PBDEs during the analysis would have been negligible.

Table 9: Concentrations of PBDEs in soil after 60 days, on a µg/kg dry weight basis (± 95% confidence interval[31]), from Huang et al. (2010)

PBDE Group[a]	BDE No.	Treatment group[b]							
		Radish	**Alfalfa**	**Squash**	**Pumpkin**	**Maize**	**Ryegrass**	**Non-spiked plant control**	**Unplanted control**
DecaBDE	BDE-209	3,073 (± 169)	4,107 (± 364)	3,402 (± 288)	4,393 (± 504)	3,612 (± 230)	3,963 (±328)	1.1-1.4	4,701 (± 390)[c]
NonaBDEs (3/3)	BDE-208	52	42.6	36.2	34.5	43.4	49.1	0.001-0.005	20.3
	BDE-207	71.6	62	63.7	55.2	62.8	80.6	0.002-0.005	30.8
	BDE-206	111.1	111.3	102.3	83.7	125.1	147.2	0.005-0.1	36.6
	Total	**234.7**	**215.9**	**202.2**	**173.4**	**231.3**	**276.9**	**0.008-0.11**	**87.3**
OctaBDEs (2/12)	BDE-197	74.8	69.0	66.1	71.2	69.3	68.2	0.004-0.009	1.1
	BDE-196	1.5	83.2	77.0	84.4	82.2	74.1	0.002-0.009	1.3
	Total	**76.3**	**152.2**	**143.1**	**155.6**	**151.5**	**142.3**	**0.006-0.018**	**2.4**
HeptaBDEs (3/24)	BDE-191	-	-	-	-	-	-	-	-
	BDE-184	-	-	-	-	-	-	-	-
	BDE-183	-	-	-	-	-	-	-	-
	Total	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
HexaBDEs (4/42)	BDE-156	-	-	-	-	-	-	-	-
	BDE-154	23.2	n.d.	n.d.	23.8	19.3	1.9	n.d.	n.d.
	BDE-153	0.8	n.d.	n.d.	43.6	n.d.	n.d.	n.d-0.3	n.d.
	BDE-138	61.3	n.d.	n.d.	nd	n.d.	4.5	n.d.	n.d.
	Total	**85.3**	**n.d.**	**n.d.**	**67.4**	**19.3**	**6.4**	**n.d-0.3**	**n.d.**

[31] 95% confidence intervals are calculated from the standard deviation data presented in the paper for decaBDE concentrations. S.d. data for the lower congeners is missing from the paper. Zhang (2010) stated that the relative standard deviation for the PBDE congeners was in the range 8 to 16%.

Table 9 (continued)

PBDE Group[a]	BDE No.	Treatment group[b]							
		Radish	**Alfalfa**	**Squash**	**Pumpkin**	**Maize**	**Ryegrass**	**Non-spiked plant control**	**Unplanted control**
PentaBDEs (5/46)	BDE-126	-	-	-	-	-	-	-	-
	BDE-119	3.6	10.8	0.2	n.d.	n.d.	n.d.	n.d.	n.d.
	BDE-100	n.d.	nd	n.d.	2.4	n.d.	0.04	n.d.	n.d.
	BDE-99	8.0	n.d.	1.0	9.1	n.d.	0.7	n.d.	n.d.
	BDE-85	20.4	9.6	8.5	9.8	22.9	22.6	0.08-0.4	0.6
	Total	**32.0**	**20.4**	**9.7**	**21.3**	**22.9**	**23.3**	**0.08-0.4**	**0.6**
TetraBDEs (5/42)	BDE-77	-	-	-	-	-	-	-	-
	BDE-71	n.d.	nd	n.d.	0.04	0.07	0.09	n.d.	n.d.
	BDE-66	0.4	0.1	1.5	0.7	0.7	1.6	n.d.	n.d.
	BDE-49	n.d.	6.9	0.09	0.2	5.9	5.1	n.d.	0.2
	BDE-47	4.7	2.3	3.2	2.2	2.3	1.4	0.06	0.4
	Total	**5.1**	**9.3**	**4.8**	**3.1**	**9.0**	**8.2**	**0.06**	**0.6**
TriBDEs (2/24)	BDE-28	2.7	2.4	3.4	2.4	3.9	4.7	n.d.	0.05
	BDE-17	-	-	-	-	-	-		
	Total	**2.7**	**2.4**	**3.4**	**2.4**	**3.9**	**4.7**	**n.d.**	**0.05**
DiBDEs (2/12)	BDE-15	-	-	-	-	-	-		
	BDE-7	0.04	0.01	1.9	1.0	0.4	n.d.	0.005-0.02	0.06
	Total	**0.04**	**0.01**	**1.9**	**1.0**	**0.4**	**n.d.**	**0.005-0.02**	**0.06**

Note: a - Figures in brackets indicate the number of isomers analysed for in the experiment against the total number of possible isomers in that congener group.

b - Values are the mean of four replicates, and after subtraction of the blank value for non-spiked soil. A dash indicates that no reported value was provided either in the paper or the supporting information file. N.d. means 'not detected'. Limits of detection are provided in the supporting information file, but generally only as a range.

c - This is the mean concentration reported in the paper. The re-calculated mean (see main text) is 4,830 µg/kg dw.

DecaBDE removal was seen in each treatment group at the end of the 60-day period, as summarized in Table 9. Further observations were as follows:

- The mean decaBDE soil concentration in the control treatment spiked with decaBDE but without plant growth was 4,960 µg/kg dw at the start of the test, and had decreased to 4,700 µg/kg dw by the end. The authors interpreted this as a loss of 5%. Zhang (2010) provided the individual measured values: 5,365.0, 4,659.0, 4,790.0 and 5,026.0 µg/kg in each of four replicates at the start; 4,967.1, 4,609.0, 4,818.5 and 4,405.9 µg/kg at the end. A two-tailed dependent *t*-test indicates that these differences are not significant at the 95% level (p = 0.18). It would therefore appear that there was no significant loss in concentration in this control. On this basis, it might be more appropriate to take the overall mean as an indication of the level of decaBDE in the unplanted control treatment. This is 4,830 [95% C.I.: 4,584 – 5,076] µg/kg dw.

- Very low concentrations of decaBDE (1.1-1.4 µg/kg dw were detected in the non-spiked soil that contained plants, which indicated that there was an uncontrolled source of the test substance in the laboratory environment. Some other PBDEs were also detected in this control group, but at much lower concentrations than were found in the tests with spiked soil. Given these low levels, this finding does not affect the validity of the experiment.

- The addition of plants appeared to have a significant effect on the transformation of decaBDE. The average loss of decaBDE (compared to the level in the unplanted control at the end of the study) was 20% (range 6-35%)[32] depending on the species. The overall pattern of lower congener formation is obscured by the fact that only a small number of isomers were determined in each congener group. The reported total concentrations for each group might therefore represent minima (although the possible co-elution of some congeners might offset this to some extent). For radish, which seems to have had the highest levels of transformation, the sum of tetra- to heptaBDEs was therefore at least 122 µg/kg dw (i.e. 2.7% of the total measured PBDE concentration present at the end of the test). The mole fractions of the tetra-, penta-, hexa-, hepta-, octa- and nonaBDE congeners in soil at the end of the radish experiment were 1.7%, 10.3%, 24.1%, 0%, 17.2% and 46.6%, respectively (ignoring the contribution from decaBDE itself)[33]. The lack of heptaBDEs is surprising.

- Several lower PBDE congeners were detected in the plant tissues. For example, although not detected in the soil[34], heptaBDEs were present at concentrations around 15-80 µg/kg dw in plants. Although the amounts were low, the proportion of pentaBDE and lower congeners (as a mole percentage) was higher in the plant samples than in soil, suggesting that further debromination occurred in the plant tissues (perhaps as a result of photodegradation) and/or the lower molecular weight substances are taken up more effectively.

[32] This is based on the mean decaBDE concentration as reported in the paper. If the re-calculated mean of 4,830 µg/kg dw is used instead, the average percentage loss becomes 22% (range 9 – 36%).

[33] For the two octaBDE congeners included in the analysis, the total concentration at the end of the test with radish was 76.3 µg/kg dw, whereas higher levels were obtained with the other plant species (up to around 156 µg/kg dw).

[34] Zhang (2010) confirmed that although two heptaBDE congeners (BDE-183 & -191) were included in the standards, neither of them was detected in the soil samples. The lack of detection of heptaBDE congeners in the soil samples is surprising, although it should be noted that only three isomers were investigated.

- The study also investigated the presence of twelve hydroxylated PBDEs in both soil and plant samples, all of which contained five bromine atoms or fewer. Three such substances were detected in plant tissues at levels up to about 100 µg/kg dw but none were found in the soil samples (limits of detection were in the range 26 to 123 µg/kg dw).

- A significant negative correlation between the residual decaBDE concentration in soil and the soil microbial biomass (measured as the total phospholipid fatty acids) (p <0.05, r^2 = 0.74) suggested that microbial metabolism contributed to the observed transformation.

The registration dossiers mark this study as valid with restrictions (Klimisch code 2), but conclude that speculation about biotransformation is erroneous because:

- Recovery of decaBDE from soil with plants was generally within the range of the matrix (soil) spike or unplanted control soil;
- Identification of any lower molecular weight PBDEs as metabolites of decaBDE is questionable given neither the composition of the test article nor the analytical results of untreated soil was provided; and
- Biotransformation over 60-days is inconsistent with other data.

The percentage removal of decaBDE is uncertain given the observed recoveries in treatments and controls. However, contrary to the statement of the registrant, results are presented for the untreated soil (as well as non-spiked plant control) in the supporting information to the paper. These show that lower molecular weight PBDEs appeared consistently in the planted treatments at higher concentrations than controls by the end of the test. The 'inconsistency' with other data is not necessarily accurate, since this statement ignores the apparent correlation with soil microbial biomass. A variety of studies have now shown that micro-organisms have the potential to degrade decaBDE in soils and sediments (see below and Section 3.1.2.2).

The role of soil micro-organism communities has been considered further by the same research group (Wang et al., 2011a). (This study was not summarised in previous EU risk assessment reports.) They performed a greenhouse rhizobox experiment with Italian ryegrass to investigate the effect of root growth and inoculation with the arbuscular mycorrhizal fungus *Glomus mosseae*[35] on the degradation of decaBDE in soil aged for four months prior to exposure. The residual decaBDE concentration was lowest in the root compartment and significantly increased up to a distance of 4 mm from the roots. Concentrations of decaBDE in the soil decreased by 20.8-56.4% and 15.3-45.3% compared to initial concentrations in inoculated and non-inoculated treatments, respectively (a loss of about 3% was observed in the unplanted control soil). The concentrations were consistently lower in the rhizoboxes inoculated with the fungus. The sum of losses due to plant uptake and adsorption to the experimental apparatus was about 0.4%, so the overall loss was therefore assumed to be caused by degradation. Significant positive correlations were also observed between biochemical measures of soil micro-organism activity and decaBDE dissipation rate. Twelve di- to nonaBDEs were detected in the soil at the end of the experiment with plants. This study seems to confirm the findings of the earlier study from the same group, and provides a possible mechanistic explanation for the observations (i.e. the degradation of decaBDE in soil is mediated by soil micro-organisms associated with plant roots).

[35] This is a representative of a group of soil micro-organisms that form a ubiquitous association with the roots of most terrestrial plant species.

In a related study, Huang et al. (2011) investigated plant uptake and dissipation of PBDEs from weathered soils from Chinese electronic-waste recycling sites. (This study was not summarised in previous EU risk assessment reports.) DecaBDE was present in the largest quantities in all soils, accounting for 35.6 – 52.8% of the total PBDEs. The soils were used in pots to grow Italian ryegrass (*Lolium multiflorum*), pumpkin (*Cucurbita pepo ssp. Pepo cv. Lvjinli*) and maize (*Zea mays cv. Nongda 108*) in a 60-day greenhouse study. A plant-free control and a control using 'PBDE-free' soil were included in the experiment. Four replicate pots of each treatment were prepared. Extraction and analysis of PBDEs in plant and soil samples used the same methods as Huang et al (2010) with minor modifications.

Eighteen PBDE congeners (tri- to decaBDE) were detected in the plant tissues at the end of the study, with twenty-one in the soils. DecaBDE was bioavailable to plants from weathered soils, since it was detected in plant roots and other tissues in all the treatments. The interpretation of the study in terms of transformation potential to specific PBDE congeners is confounded by the presence of other PBDEs in the starting soil, and possible presence of other substances that could interfere with the analysis. However, the results show that planting significantly ($p < 0.05$) enhanced dissipation of PBDEs in the soils compared with the plant-free controls: the total PBDE concentration in the soils had reduced by 13.3 to 21.7% after harvest. Plant uptake was estimated to have contributed to 0.36 – 0.92% of the loss, with a further 0.07% due to adsorption to the pots themselves. A significantly positive correlation was obtained between the reduction in PBDEs in the soils (plant-free treatment included) and the soil microbial biomass measured in terms of total phospholipid fatty acids ($r^2 = 0.77$, $p < 0.01$), confirming that microbial metabolism and biostimulation of microbial communities by planting are important contributors to the dissipation of PBDEs in soil.

The ratio of concentrations in above-ground versus root tissue was 0.18, 0.27, 0.83 and 0.31, for tetra-, penta-, nona- and decaBDE, respectively. This ratio reflects the combined contribution of both root-to-shoot translocation and any metabolism of PBDEs inside the plants. The nonaBDEs had higher ratios than other PBDEs. It was also noted that the final soil concentration of two nonaBDEs (BDE-206 and -207) had increased by 1.2 – 3.9 times compared with their initial concentrations, which could be due to debromination of decaBDE in the soils.

This experiment has similar methodological limitations as the Huang et al. (2010) study, and it is noted that the observations were not made by researchers who were independent of that study. Nevertheless, this experiment provides further supporting evidence for the importance of plants in PBDE removal.

A further study by this research group (Zhao et al., 2011 and Wang et al., 2011b) investigated the uptake, translocation and debromination of three lower molecular weight PBDE congeners (BDE-28, -47 and -99, a tri-, tetra- and pentaBDE, respectively) in a hydroponic experiment using maize (*Zea mays* cv. Zhengdan 1). Although not directly relevant to decaBDE, it is of interest because it was found that debromination products were detected in all parts of the plant, which suggests that the plants themselves might perform this reaction.

Independent from the Chinese research group, Vrkoslavová et al. (2010) investigated the accumulation and translocation of PBDEs by tobacco (*Nicotiana tabacum*) and nightshade (*Solanum nigrum*). Plant seedlings were planted in pots containing PBDE-contaminated sewage sludge (collected from a wastewater treatment plant in Hradec Králové, Czech Republic) as well as an uncontaminated garden substrate used as a control. Unplanted sludge was also used as a further control. Five pots were used per treatment, except for the garden substrate controls, where three pots were used for each plant species (six pots in total). DecaBDE was the predominant congener (400.3

ng/g) in the original sewage sludge. Lower molecular weight PBDE congeners were present at a total concentration of 376.5 ng/g, as follows: BDE-47 (139.4 ng/g), BDE-99 (166.3 ng/g) BDE-100 (28.7 ng/g), BDE-28 (1.1 ng/g), BDE-49 (9.9 ng/g), BDE-66 (3.3 ng/g), BDE-85 (6.6 ng/g), BDE-153 (9.1 ng/g), BDE-154 (8.9 ng/g) and BDE-183 (3.2 ng/g). The paper does not indicate how long the sludge was aged prior to introduction of the plants. The pot volume was 200 ml and they were lined with aluminium foil to prevent sorption of PBDEs from sewage sludge onto the pots and to prevent outflow of water from pots. Pots were maintained at 25°C and were regularly watered for 6 months. The plants in the control substrate were fertilized periodically after 6 weeks.

PBDEs were analysed by GC/MS-NCI and suitable quality control measures were taken. The total PBDE concentration, as well as individual PBDE congener concentrations in the growth media, did not change significantly after 6 months of cultivation in any of the treatments (both in the unplanted sewage sludge and in the sewage sludge planted with tobacco or nightshade). After 6 months, the amount of decaBDE present in the unplanted sludge was 34,600 ng, whereas the amount in the sludge with nightshade was 45,400 ng, and the amount in sludge with tobacco was 46,300 ng. It is unclear why the amounts were so different between planted and unplanted sludge (no indication of measurement precision or accuracy for these particular results is given in the paper).

Plants grown in sewage sludge were more robust and had a higher amount of biomass. After 6 months of plant cultivation in the contaminated sewage sludge, up to 15.4 ng/g dw and 76.6 ng/g dw of three PBDE congeners (BDE-47, -99 and -100) were accumulated in the nightshade and tobacco tissue, respectively. Concentrations in control plants were ten times lower. The majority of the PBDEs was detected in aboveground plant biomass indicating that both plants have the ability to translocate PBDEs. DecaBDE accumulated only in tobacco plants, at a concentration of 116.8 ng/g dw.

The homogeneity of the PBDE distribution in the sludge was not indicated, and there is no information on the influence of ageing on the results. Compared to the study of Huang et al. (2010), the test concentration of decaBDE was an order of magnitude lower, the plant species were different (they were chosen for their known ability to accumulate polychlorobiphenyls) and the exposure duration substantially longer, so the results are not directly comparable. In particular, this study used a contaminated sludge with a mixture of PBDEs, rather than soil dosed with decaBDE alone. Whilst sludge might offer a more relevant exposure route than direct addition to soil, the microbial communities and decaBDE bioavailability will differ. In addition, this study would not be able to distinguish small concentrations of any metabolic products from the native PBDEs, and hydroxylated PBDEs were not investigated. Whilst therefore not providing any evidence of transformation, this study does not necessarily contradict the findings of Huang et al. (2010) either.

Other studies

Indirect evidence of decaBDE's overall persistence in soils is provided by monitoring studies in agricultural fields several years after the last known application of contaminated sewage sludge (e.g. Sellström, 2005; Sellström et al., 2005; and Eljarrat et al., 2008). However, since these studies provide no information on degradation products, they are not summarised here.

1) A simulation test following standard OECD test guidelines is not available. However, Nyholm et al. (2010b) investigated the primary biodegradation kinetics of decaBDE (along with other brominated flame retardants) in soil in laboratory microcosms incubated at 20°C over 120-160 days (i.e. up to about five months). (This study was not summarised in previous EU risk assessment reports.) The substance was mixed with three types of sewage sludge (activated, digested and 'hygienized' (i.e. sterilized

by heat treatment)) before each sludge was mixed with a heavy clay agricultural soil at a level of 0.5% w/w on a dry weight basis. The experimental method was similar to OECD Test Guideline 307 (Aerobic and Anaerobic Transformation in Soil), the major difference being that only one type of soil was tested. No information is given about the organic carbon content of the soil in the paper, but other sources indicate that it is about 2.1%[36].

The nominal decaBDE concentration in the treated soil was not reported explicitly, but was in the range 40 – 70 ng/g dw. No, or 'relatively little', degradation was observed in the controls (the supplementary data indicate that the decaBDE concentration declined from 31 to 29 ng/g dw over the 160-day period in the autoclaved anaerobic soil control). The graphs presented in the paper indicate that the decaBDE concentration declined by almost 20% in the anaerobic test after 160 days, with a roughly similar decline under aerobic conditions over 120 days. The authors stated that this decline was not statistically significant, and Nyholm (2011) provided further data to indicate that this was due to the high relative standard deviation observed (up to 15% in the triplicate samples) (Table 10).

Table 10: Concentration trends of decaBDE in sludge-amended soil, from Nyholm (2011)

Incubation time (days)	**DecaBDE concentration (ng/g soil dw)**		
	Aerobic soil (digested sludge)	**Aerobic soil (active sludge)**	**Anaerobic soil (active sludge)**
0	54±1	44±7	60±9
4	49	-	52
7	47±4	45	57±3
10	-	-	53
14	44	42	-
30	47±5	53	49
60	43	45	-
70	-	-	55
90	48±7	44±5	-
120	44	43	52
160	-	-	50

Note: Values are average ± standard deviation (n = 3)

The extrapolated primary degradation half-life under both aerobic and anaerobic conditions was >360 days (assuming exponential decay).

This study implies that the degradation rate of decaBDE in this soil type is slow under both aerobic and anaerobic conditions. This might be related to the application method of the test substance to the soil (i.e. adsorbed to sewage sludge), which could have limited its bioavailability to micro-organisms. The variability in analytical measurements makes it difficult to draw clear conclusions about the actual rates, but it does not preclude the possible formation of small amounts of degradation products, which were not investigated. Plants were not present in the soils, so this experiment represents different conditions to those used by Huang et al. (2010).

2) Liu et al. (2011) examined the effects of decaBDE on soil microbes. (This study was not summarised in previous EU risk assessment reports.) The soil was a silty clay loam (9.15 g/kg organic matter, pH 7.69, 11.50 CEC/cmol/kg) collected from Zijin Mountain in Nanjing, China. The soil was thoroughly mixed and stones, large plant residues, and macrobiota were removed. One kilogram of sieved soil (<5 mm) was

[36] The soil was one of thirteen Nordic reference soils, from Lanna in Sweden. Details are provided at, for example, http://www.tidsskrift.dk/visning.jsp?markup=&type=cont&id=72131&query=changes&journal=all&fromyear=&toyear=&m=25&n=100.

spiked with decaBDE (purity not stated) by direct addition, to achieve three final decaBDE concentrations of 1, 10, and 100 mg/kg (dry weight). Untreated controls and the spiked soils were ground (<2 mm) to achieve an even distribution within the samples. A 1 kg soil sample (dry weight basis) for each replicate was weighed into an incubation vessel. All soil for the treatments and controls were obtained from the same site, and all treatments and controls were incubated at 25 °C and kept in complete darkness. Soil samples were taken for further analysis at 15, 30, 60, 90, 120, 150 and 180 days after treatment. Each treatment was replicated three times.

Sampled soil (10 g) was freeze-dried and ground then Soxhlet extracted with a mixture of acetone and hexane (1:1, v:v) for 48 hours. Concentrated extracts were cleaned and fractionated on a 1 cm silica/alumina column. DecaBDE was eluted with 30 mL of hexane and 60 ml of hexane:methylene chloride (1:1), with the final extract volume being 1 ml. Samples were analyzed using high performance liquid chromatography. Procedural and spiked blanks were processed alternately throughout the sampling analysis. No decaBDE was found in the procedural blanks, and recoveries ranged from 86.4 to 102.5% in the spiked blank samples.

No degradation of decaBDE was observed after 180 days (actual concentrations are not reported; analysis for lower molecular weight PBDE congeners was apparently not performed). The method of dosing might have limited the availability of the substance to the micro-organisms. Measurements were made of soil microbial activity and community structure using denaturing gradient gel electrophoresis, fluorescence *in situ* hybridization and soil enzymatic activity analyses. *Pseudomonas*, *Bacillus* and uncultured bacteria dominated the bacterial communities in all soil treatments. Principal component analysis showed that high doses of decaBDE altered the soil microbial community structure, and inhibited total cell growth in the soil microcosms as well as the cell counts of the α-,β-,γ-proteobacteria subgroups and the *Cytophaga–Flavobacterium–Bacteroides* groups. The suppression effect increased as the decaBDE concentration increased. The application of decaBDE also elevated and then inhibited urease and alkaline phosphatase activities.

3) Zhou et al. (2007) investigated the degradation of decaBDE (98% purity) by white rot fungi under aerobic conditions. (This study was not summarised in previous EU risk assessment reports.) Cultures were prepared by adding 1 ml of a solution of decaBDE in dichloromethane (concentration 160 mg/l) to a 250 ml flask and allowing the solvent to evaporate (this produced a coating of 160 µg of decaBDE on the bottom of the flask). 100 ml of an aqueous culture medium (containing yeast, peptone and glucose) was added and the flasks were inoculated with white rot fungi GIM3.383 (the amount added was not reported). The flasks were then incubated in the dark for up to 10 days at 30°C on an orbital shaker. The experiment was carried out in triplicate and sterile samples were also prepared as controls.

   The amount of decaBDE present in the flask was found to decrease from an initial (nominal) value of 160 µg to 92.5 µg over 10 days (42.2% degradation). No significant change in the amount of decaBDE in the control cultures occurred over the same timeframe.

   Experiments were also carried out to investigate the effects of a non-ionic surfactant (Tween 80) and β-cyclodextrin on the bioavailability of decaBDE in this system. The culture flasks were prepared in the same way as the main experiment, with the addition of either Tween 80 or β-cyclodextrin at concentrations up to 900 mg/l. These two substances increased the extent of decaBDE removal:

   - 96.5% removal of decaBDE occurred in 10 days at a Tween 80 concentration of 500 mg/l (and no decaBDE could be detected after 12 days). Removal

appeared to be inhibited at higher surfactant concentrations (e.g. removal was 31.5% after 10 days at a Tween 80 concentration of 900 mg/l).

- β-Cyclodextrin showed a similar, but less marked, enhancement of the removal of decaBDE. The highest removal rate (78.4% after 10 days) occurred at a β-cyclodextrin concentration of 700 mg/l. A slightly lower degradation rate (76.4% after 10 days) occurring at a β-cyclodextrin concentration of 900 mg/l.

No analysis for degradation products was performed. It should be noted that the mass of mycelium present in the cultures increased during the course of the experiment, and the increase in the mass of the mycelium in the presence of both Tween 80 and β-cyclodextrin followed a broadly similar trend to the removal rate. Although the analytical method included a step for the extraction of decaBDE from the mycelium, no information is given in the paper as to the effectiveness of the extraction method used (sonication for fifteen minutes with dichloromethane). Therefore it is possible that some of the apparent loss of decaBDE seen in this study was caused by adsorption to the mycelium (and incomplete extraction during the analytical method).

This study therefore provides equivocal evidence of degradation, but is interesting because of the apparent role of fungi in the degradation of decaBDE in soil experiments (see above).

4) He et al. (2006) studied the biodegradation of decaBDE by cultures of anaerobic bacteria. The test substance had a purity of >98%. Experiments were also carried out using a commercial octaBDE product (with a reported purity of >98%; it contained two nonaBDE congeners, three octaBDE congeners, two heptaBDE congeners and one hexaBDE congener). The bacteria used in the study included *Dehalococcoides ethenogenes* 195 (a strain that had previously been shown to have ability to dechlorinate chloroethanes, chlorobenzenes and polychlorinated dibenzo-*p*-dioxins), *Dehalococcoides* sp. strain BAV1 (that had previously been shown to be capable of dehalogenating dichloroethanes, vinyl chloride and vinyl bromide), an enriched autotrophic culture containing *D. ethenogenes* 195, an enrichment containing a number of *Dehalococcoides* spp. and *Sulfurospirillum multivorans* (that had previously been shown to be capable of dechlorinating tetrachloroethene to dichloroethene).

The microorganisms were grown in 160 ml serum bottles containing 50 ml bicarbonate-buffered mineral salts medium and 0.2 mM l-cysteine and 0.2 mM Na_2S. The headspace of the bottles was filled with a mixture of hydrogen and carbon dioxide (80:20 v/v) except for the experiments with the enrichment culture containing a number of *Dehalococcoides* spp., where a mixture of nitrogen and carbon dioxide (80/20 v/v) was used. Acetate (5 mM) was used as the carbon source in the experiments with pure cultures, whereas lactate (10 mM) was used as the carbon source in the experiments with *Dehalococcoides* spp. No carbon source was used with the autotrophic culture containing *D. ethenogenes* 195. The bottles were sealed with rubber septa and aluminium crimp caps to ensure anaerobic conditions were maintained and then autoclaved for 25 minutes at 121°C prior to inoculation.

At the start of the experiment, the test substance was added to the bottle as a solution in trichloroethene. The initial concentrations in the bottles were 0.1 µM for decaBDE or 1.3 µM for octaBDE, and each bottle also contained 1 mM of trichloroethane. The bottles were then inoculated with active cultures growing on ~ 500 µM trichloroethane (or vinyl chloride in the case of *Dehalococcoides* sp. strain BAV1) at 5% or 10% v/v. Abiotic control bottles were also prepared. All samples were incubated in the dark at 30°C without shaking. Samples of the culture medium

were taken at weekly intervals during the experiment and analysed for the presence of polybrominated diphenyl ethers. Each experiment was carried out using duplicate biological samples, and was repeated at least once to confirm the results.

In the experiments with *S*. *multivorans*, the trichloroethylene present in the system was found to be completely dechlorinated to cis-dichloroethylene within one week. No degradation of either decaBDE or octaBDE was evident over this one week period. However, after 2 months incubation, decaBDE was no longer detectable in the culture, and octa- and heptaBDE congeners had appeared in both replicates. No degradation of decaBDE was evident in the controls. In contrast to this, no degradation was evident in the experiments with *S*. *multivorans* using octaBDE, even after incubation for one year.

No degradation of decaBDE was seen in the experiments using *D*. *ethenogenes* 195 when incubated for up to one year. This system was, however, found to debrominate octaBDE to hepta-, hexa- and pentaBDE congeners within six months, with tetraBDE congeners being formed after six months. Similar products were also formed from octaBDE using the enriched autotrophic culture of *D*. *ethenogenes* 195 but here the degradation appeared to occur at an enhanced rate (degradation was detectable after 10 weeks incubation, probably reflecting the higher cell numbers present in this enrichment culture). No debromination of decaBDE occurred with this enrichment culture.

The experiments with *Dehalococcoides* sp. strain BAV1 showed no degradation of either decaBDE or octaBDE over one year. However, when this strain was added to the enrichment autotrophic culture of *D*. *ethenogenes* 195 with octaBDE, debromination to tetra- and dibrominated diphenyl ether congeners was evident after 3 months incubation.

No degradation of decaBDE or octaBDE was evident in the experiments using an enrichment containing a number of *Dehalococcoides* spp. after one year. This culture was known to not contain either strain 195 or strain BAV1. However, when strain 195 was added to this culture, debromination of octaBDE was evident after three months incubation, with hepta- to diBDE congeners being formed.

Further experiments were carried out to investigate if the trichloroethylene present in the system either promoted or inhibited the debromination. In these experiments, the test substance was dissolved in nonane and this solution was added to bottles containing either the *D*. *ethenogenes* 195 culture or the enriched autotrophic culture of *D*. *ethenogenes* 195. No debromination was seen in any of these experiments after incubation for one year. The authors concluded that the debromination seen in these cultures required the presence of electron acceptors other than the PBDEs themselves.

In summary, this reliable study shows that decaBDE can be biodegraded by some strains of anaerobic bacteria. In some cases, debromination to heptaBDE congeners was evident after 2 months' incubation at 30°C. Experiments with octaBDE showed that debromination can proceed to hexa-, penta- and tetraBDEs after around six months. The reaction apparently required the presence of electron acceptors other than the PBDEs themselves (i.e. debromination occurred cometabolically).

5) Robrock et al. (2008) investigated the debromination pathways of seven individual PBDE congeners by three different cultures of anaerobic dehalogenating bacteria. The objective of this study was identification of debromination pathways rather than

the calculation of biotransformation kinetics[37]. (This study has not been summarised in previous EU risk assessment reports.)

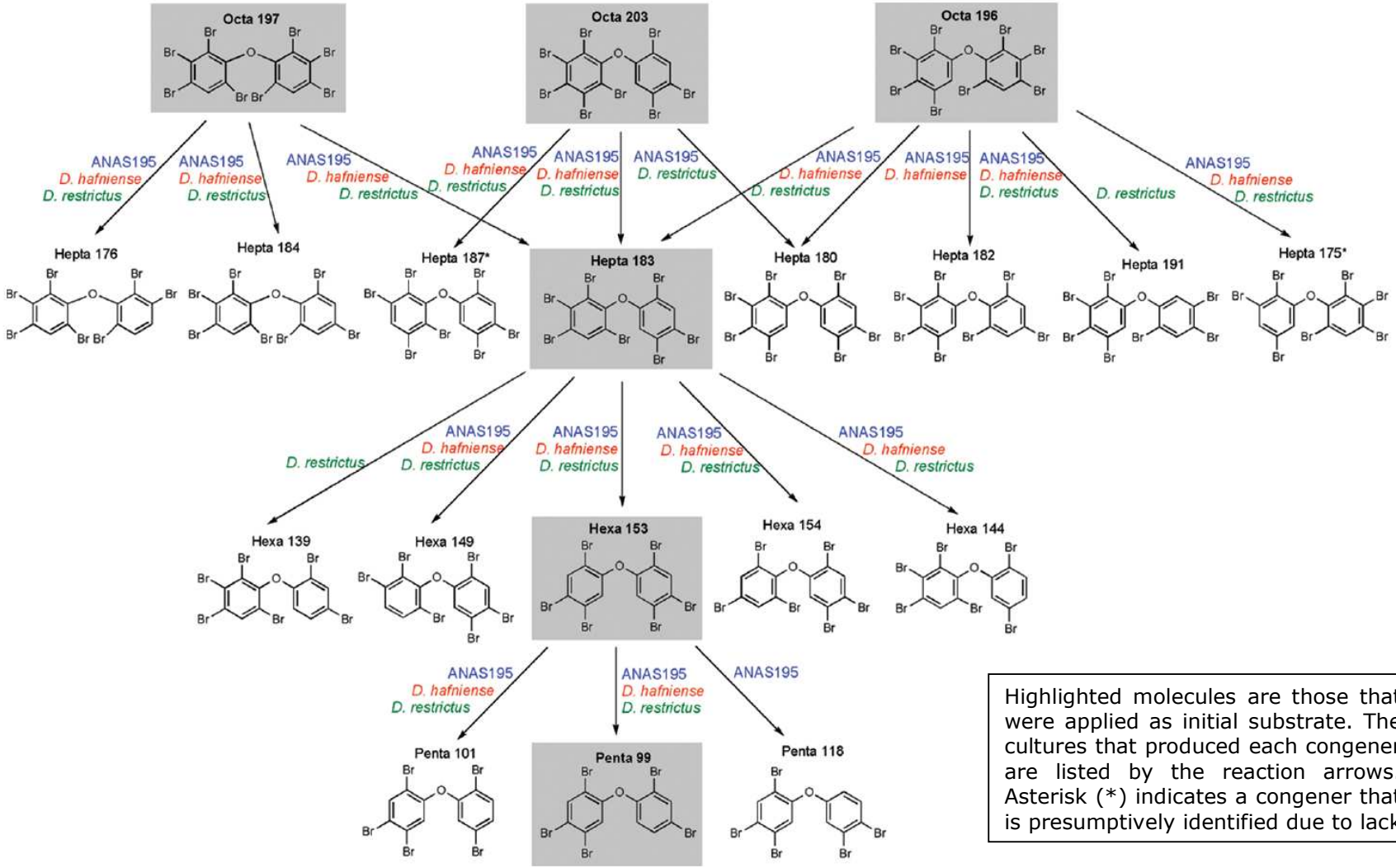
The dehalogenating cultures evaluated in this study were a trichloroethene-enriched consortium containing multiple *Dehalococcoides* species (ANAS195), and two pure cultures, of *Dehalobacter restrictus* PER-K23 and *Desulfitobacterium hafniense* PCP-1. These were grown in mineral salt medium with different carbon sources, and either perchloroethene (PCE) or pentachlorophenol. The congeners that were synthesised were the five major components of the commercial octaBDE product (BDE-196, -197 and -203 (three octaBDEs), BDE-183 (a heptaBDE), and BDE-153 (a hexaBDE)), as well as BDE-99 and BDE-47 (a penta- and tetraBDE, respectively). Individual PBDE congeners dissolved in nonane were added to the test medium to achieve a final concentration of 20 µg/l (25 nM for the octaBDEs, 27 nM for heptaBDE, 30 nM for hexaBDE, 35nM for pentaBDE, and 40nM for tetraBDE). All bottles contained resazurin as an oxygen indicator. Active or autoclaved cultures were inoculated after addition of the PBDEs. Uninoculated abiotic controls were used for the spore-forming *Des. hafniense* as original autoclaved controls for this culture revived during incubation. All samples and controls were incubated at 30°C in the dark without shaking. Experiments were conducted with triplicate biological samples and single controls and were monitored for three months. Most experiments were repeated for verification of the results.

Samples were removed each month and extracted for PBDE analysis by comprehensive two-dimensional gas chromatography (GC x GC) coupled to an electron capture detector to maximize separation and identification of the product congeners. Except for two unavailable congeners, all substrate and product PBDE peaks were matched with standards for specific identification and quantification (but mass balance was not calculated).

All tested PBDE congeners were found to be transformed by all cultures over three months of incubation. The debromination pathway for each congener was similar for all tested cultures, with exceptions typically represented by a single congener produced in trace quantities, although the extent of debromination varied greatly between cultures and congeners. Figure 4 summarizes the observed debromination pathway for all tested congeners (for simplicity, it was assumed that there was no isomeric rearrangement of bromines around the PBDE ring; although Figure 4 depicts only the products of one bromine removal for each tested congener, further debromination products were frequently detected but it was not possible to determine which parent PBDE was involved).

[37] Zeng et al. (2010) developed a model based on carbon-bromine atom dissociation energies to predict the transformation of several PBDE congeners (BDE-203, -197, -196, -153, and -47). Following comparison with experimental values of reactions with light, anaerobic micro-organisms (using the results of Robrock et al., 2008) and zero-valent iron, the authors claim that the model can be used to predict the major debromination products for any PBDE congener. This paper appears to be based on a number of laboratory studies of limited environmental relevance, so has not been reviewed in any detail for the purposes of this dossier.

Figure 4: PBDE debromination pathways, taken from Robrock et al. (2008)



Highlighted molecules are those that were applied as initial substrate. The cultures that produced each congener are listed by the reaction arrows. Asterisk (*) indicates a congener that is presumptively identified due to lack

In some cases, due to the synthesis process, the substrates contained impurities that were also possible debromination products. For example, BDE-203 contained trace (around 0.1 mol percent) quantities of two heptaBDEs, and BDE-183 contained two hexaBDEs. However, in each case these PBDEs were confirmed as being biologically produced because the concentrations increased significantly (at least two-fold) in the active bottles while remaining constant in control bottles during the three month incubation.

Biotransformation of the octaBDE congeners was slow and limited for all tested cultures. For example, BDE-197 was debrominated to heptaBDEs (BDE-183 by ortho- substitution, BDE-184 by meta- substitution and BDE-176 by para-substitution) by all three cultures. All congeners were generated in approximately equal quantities, cumulatively amounting to 9 mol per cent or less of the total recovered molar concentration of PBDEs at the end of three months.

The heptaBDE (BDE-183) was debrominated relatively rapidly by all three cultures, producing many products including four hexaBDEs. A further hexaBDE product was produced by *D. restrictus*.

The hexaBDE (BDE-153) was debrominated to two pentaBDEs (BDE-99 and -101) by all three cultures. A further pentaBDE congener (BDE- 118) was produced by one culture in trace quantities.

In all cultures, the pentaBDE (BDE-99) was debrominated to two tetraBDEs (BDE-47 and -49) by meta- and para- substitution respectively. ANAS195 and *Des. hafniense* also produced BDE-66, whereas *Des. hafniense* and *D. restrictus* produced BDE-48. BDE-49 and -48 were the predominant congeners, representing up to 22 mol per cent, whereas BDE-47 and -66 were detected at concentrations less than 1 mol per cent after three months.

When the tetraBDE (BDE-47) was exposed to bacteria as a substrate, it was biotransformed relatively rapidly particularly in the *Des. hafniense* and *D. restrictus* cultures, with almost complete conversion to a triBDE (BDE-17), which was quickly debrominated further to diBDE (BDE-4) as a major product (28 and 83 mol per cent in the *Des. hafniense* and *D. restrictus* cultures, respectively). It is possible that further debromination products such as monoBDEs were produced in these cultures, but these congeners were never detected, perhaps due to the low sensitivity of electron capture detection to monoBDEs, interference with compounds in the bacterial medium that elute at the same time as mono-BDEs, and low monoBDE concentrations. The ANAS195 culture produced in addition BDE-28 in trace concentrations (0.2 mol per cent), and BDE-4 was not observed.

To determine whether chlorinated substrates were required to induce PBDE debromination, additional experiments were conducted with the three cultures exposed to a commercial octaBDE product in the absence of PCE or pentachlorophenol. No debromination was observed in the *D. restrictus* and *Des. hafniense* samples, whereas debromination was observed in the ANAS195 culture. These results suggest that either the debrominating enzymes were not induced by the PBDEs alone or that the PBDE transformation by these isolates is cometabolic, requiring the concomitant presence of energy-generating electron acceptors. In contrast, the ANAS195 culture was able to debrominate PBDEs in the absence of PCE, although the mechanism behind this reaction is unclear.

All cultures exhibited preferences for removing bromines at certain positions, typically the meta- and para- bromines, often with multiple para- or meta- bromine removal products being formed. The most commonly substituted bromines were those that are double flanked, which is likely to be due to their high enthalpies of formation given the repulsion between adjacent bromine atoms. Ortho- bromines were also frequently removed, although typically the products were minor ones.

6) Robrock et al. (2009) exposed four bacterial isolates to thirteen PBDE congeners ranging from mono- to hexaBDEs at part per billion levels for three days under aerobic conditions at 30°C. (This study has not been summarised in previous EU risk assessment reports.) The four strains were two PCB-degrading bacteria, *Rhodococcus jostii* RHA1 and *Burkholderia xenovorans* LB400; a related strain known to degrade aromatics, *Rhodococcus* sp. RR1; and an additional ether degrading bacterium, *Pseudonocardia dioxanivorans* CB1190. RHA1 and LB400 were initially grown on biphenyl, whereas RR1 and CB1190 were grown on pyruvate and 1,4-dioxane, respectively.

   The percentage PBDE transformation was determined by comparing the PBDE concentrations remaining in the live samples with those present in the autoclaved controls in order to account for extraction efficiencies and any potential mass losses. The two PCB-degrading strains transformed greater than 90% of the mono- and diBDE congeners within three days, but only 10-45% of individual pentaBDE congeners. When exposed to a commercial pentaBDE product (DE-71) RHA1 transformed 95% of BDE-47 (a tetraBDE), 78% of BDE-99 and 45% of BDE-100 (both pentaBDEs). HexaBDEs were found to be the most resistant to transformation by these strains: of the three tested congeners, LB400 was only able to transform 18% of BDE-138 but not BDE-153 or -149. RHA1 was unable to degrade any of the three tested hexaBDE congeners even at increased cell densities.

   RHA1 released stoichiometric quantities of bromide while transforming mono- and tetraBDE congeners. In contrast, LB400 converted most of a monoBDE to a hydroxylated monoBDE.

   Rhodococcus sp. strain RR1 transformed monoBDE and BDE-7 (2,4-diBDE), but was unable to transform other congeners including BDE-4 (2,2'-diBDE). It therefore appears that RR1 can only transform PBDEs with one non-brominated ring. CB1190 transformed only about 16% of the monoBDE and none of the more highly brominated congeners.

   In general, transformation of the mono to hexaBDE congeners by these bacteria was inversely proportional to the degree of bromination, and the authors suggested that this was due to increasing molecular size and hydrophobicity, both of which reduce availability to the cell. Increased bromination also decreases the susceptibility of the carbons to hydroxylation and can sterically hinder enzymatic attack.

7) Lee and He (2010) established microcosms with soils and sediments from 28 locations (from China, Singapore and the USA) to determine their debromination potential with a commercial octaBDE product consisting of hexa- to nonaBDEs. This study is summarised in Section 3.1.2.2, and the conclusion was that micro-organisms dwelling in natural environments from a range of locations are able to debrominate octaBDEs anaerobically in a matter of weeks, with the formation of hexaBDEs even in circumstances where no electron acceptors were added. The temperature of the study is not typical of soils, but this may simply mean that the reaction will be slower under normal conditions.

Discussion

A simulation study of soil biodegradation at 20°C found that the mean decaBDE concentration declined by almost 20% under anaerobic conditions after 160 days, with a roughly similar decline under aerobic conditions over 120 days (Nyhom et al., 2010b). However, due to high variation amongst replicates, this decline was not statistically significant. The extrapolated primary degradation half-life under both aerobic and anaerobic conditions was >360 days (assuming exponential decay). This might be related to the application method of the test substance to the soil (i.e. adsorbed to sewage sludge), which could have limited its bioavailability to micro-organisms.

Although not performed in accordance with GLP or any standard test guideline, the Huang et al. (2010) study shows that inclusion of plants seems to have a significant effect on the transformation of decaBDE, forming tetra- to hexaBDE congeners at a decaBDE soil concentration of about 5 mg/kg dw (which is consistent with measured levels in European field situations[38]). An average loss of decaBDE of 20% (range 6-35%) was observed over a two-month period, although there are some uncertainties in these values related to the ability of the analytical procedure to recover the substance from soil. For radish, which seems to have led to the highest levels of transformation, at least 122 µg/kg dw of tetra- to heptaBDEs were formed (i.e. 2.7% of the total measured PBDE concentration present at the end of the test). The mole fractions of the tetra- to nona-BDE congeners at the end of the radish experiment were 1.7%, 10.3%, 24.1%, 0%, 17.2% and 46.6%, respectively (ignoring the contribution from decaBDE itself). Uptake into the plants was observed, and some hydroxylated PBDEs were also detected. Huang et al. (2011) made similar observations using a contaminated soil from an industrial location, which provides further supporting evidence for the importance of plants in PBDE removal.

The observations appear to be related to the soil microbial biomass, and in particular mycorrhizal fungi associated with root growth (Wang et al., 2011a). There are no other directly comparable studies on decaBDE in independent laboratories with which to compare the results[39]. Vrkoslavová et al. (2010) performed a similar study using two different plant species grown in contaminated sewage sludge for six months, and did not detect any reduction in decaBDE concentration. The decaBDE concentration in this study was an order of magnitude lower than in Huang et al. (2010) and the sludge was also contaminated with other PBDEs. It might not therefore have been possible to distinguish a low level of PBDE formation in this study, and microbial conditions and bioavailability would differ from soil treatment alone. In addition, other studies have shown that fungi and both aerobic and anaerobic bacteria found in soils (and sediments) have the potential to degrade decaBDE and other highly brominated PBDEs, and in some cases can form hepta- and hexaBDEs after several weeks' incubation at 30°C (e.g. Zhou et al.,

[38] For example, Sellström et al. (2005) found that levels in a farm soil were still of the order of milligrams per kilogram dw around 20 years after the last known input of contaminated sewage sludge.

[39] A 21-day plant toxicity test was reported in EC (2002), but it was assumed that soil concentrations were constant and no measurements were taken at the end of the test.

The fate of lower molecular weight PBDE congeners in soil including plant exposure was investigated by Mueller *et al*. (2006). A silty clay loam soil (1.8% organic matter) was spiked with a commercial pentaBDE product dissolved in acetone, to give a final concentration of 75 µg/kg. Solvent was allowed to evaporate, and the soil was then tumbled for two hours. Radish *Raphanus sativus* and summer squash *Cucurbita pepo* seeds were introduced within 24 hours of the soil amendment, with ten replicates per treatment. Pots were maintained in a controlled growth room (20°C, 8 h dark/16 h light cycle, 520 lux) for 10 weeks, and were brought up to 75% of the water holding capacity daily. The extractability of three congeners (BDE-47, -99, and -100) was monitored in planted and unplanted treatments. These three components were chosen because of analytical sensitivity constraints, but they accounted for >95% of the total PBDE concentration in the soil. Parallel aging studies were set up using soil that was either sterilised with an autoclave, or kept dry (~2% moisture content), and both of these treatments were stored in the dark for the duration of the plant tests.

The extractability of each congener decreased rapidly in the experimental soil. Total recovered PBDE concentrations were below 5 µg/kg in the unplanted, radish monoculture and squash monoculture treatments at the end of the experiment (a 90% decrease from initial levels). This was believed to be due to abiotic sorption to soil particles, since a similar low recovery was also observed in the sterilized and dry soils. PBDE recovery from mixed species plantings was nearly eight times higher than that of unplanted and monoculture treatments, indicating that inter-specific plant interactions may enhance PBDE bioavailability in soil. Evidence for competitive interactions between the two species was revealed by reduced shoot biomass of squash plants in mixed treatments relative to pots containing squash alone. The differences in PBDE soil concentrations were not driven by root biomass alone. It was noted that competition between plants is known to alter root exudate production, which in turn may affect soil microbial communities. Unfortunately, the study did not specifically investigate whether degradation might have occurred, although the authors noted that they found no evidence of any plant enhancement of PBDE dissipation. A major difference from the decaBDE study described in the main text is that the treated soil was not allowed to age prior to introduction of the plants.

2007; Lee & He, 2010). These studies cannot be directly related to actual reaction rates in the environment due to the conditions used (e.g .single strains, temperature, etc.), but they provide evidence of the capability of various microbes to transform PBDEs. The reaction appears to be easier for octaBDEs than decaBDE, with hepta-, hexa- and pentaBDE congeners formed within six months. The reaction can occur in both the presence and absence of electron acceptors other than the PBDEs themselves, and several species seem capable of performing it. In addition, the role of arbuscular mycorrhizas in pollutant degradation has been independently demonstrated with other substances[40].

As for sediment, a number of other factors must be taken into account in extrapolating the findings of any single study to the wider environment:

- It is possible that degradation kinetics might vary with soil loading. No information is available on this.
- The degree of adaptation of the micro-organisms to PBDE (or other halogenated substance) exposure, and community structure, might be important.
- Soil is a heterogenous matrix, and it is likely that the soil matrix and composition can significantly affect the availability of decaBDE to micro-organisms (or for other processes)[41].
- The soil spiking technique might also play a role in bioavailability. The influence of sewage sludge application on degradation rate is unknown.
- The influence of temperature is unknown. The Huang et al. (2010) study used a 25/20°C day/night temperature regime; a summer soil temperature of around 8-16°C might be more relevant for temperate climates.
- It is not known what degradation rate might be observed with other plant species.

Since the plant studies were not carried out under field conditions, and used a method of spiking that does not mimic the typical entry route of decaBDE to soil (i.e. adsorbed to sewage sludge), it is possible that degradation rates under natural conditions would be slower than those observed. However, the soil was allowed to age, which might mitigate this omission to some extent, and decaBDE has been shown to be bioavailable in

[40] For example, Joner et al. (2001) performed a laboratory experiment with clover and ryegrass grown on soil spiked with anthracene (500 mg/kg), chrysene (500 mg/kg) and dibenz(a,h)anthracene (50 mg/kg). Dissipation of condensed PAHs was enhanced in the presence of arbuscular mycorrhiza, with reductions of 66% and 42% in chrysene and dibenz(*a*,*h*)anthracene concentrations, respectively (compared with 56% and 20% reductions in non-mycorrhizal controls, respectively). Addition of a surfactant accelerated initial PAH dissipation but did not attain final PAH concentrations below those obtained with non-mycorrhizal plants. Toxicity tests (earthworm survival and bioluminescence inhibition in *Vibrio fischeri*) indicated that mycorrhiza reduced the toxicity of PAHs and/or their metabolites and counteracted a temporally enhanced toxicity mediated by surfactant addition. Phospholipid fatty acid profiles demonstrated that the treatments altered the microbial community structure and indicated that the mycorrhiza-associated microflora was responsible for the observed reductions in PAH concentrations in the presence of mycorrhiza.

[41] For example, Nyholm et al. (2010a) found that the use of different soils (artificial OECD soil and two natural Swedish soils) affected the degree of accumulation of several lower PBDEs in earthworms (Eisenia fetida). This was considered to be due to differences in organic matter and mineral content and therefore partitioning, although growth dilution might have been partly responsible. They did not present results for decaBDE for this portion of the experiment (possibly due to the low level of accumulation that was observed). The study also found that soil aging resulted in decreased accumulation of PBDEs with six or fewer bromine atoms, but did not affect accumulation of BDE-183 (a heptaBDE) or decaBDE, possibly due to their lower mobility in soil.

contaminated soil collected from industrial sites[42]. It should also be noted that higher concentrations of the various congener groups may have been present than were reported, due to the limited chemical analysis.

Whilst several questions remain about decaBDE's soil degradation kinetics, the Huang et al. (2010) study is a good quality study, appears to be environmentally relevant and there is no obvious reason to doubt the findings (indeed there is a plausible explanation for the observations, i.e. micro-organism communities associated with plant roots may play an important role). In the absence of contradictory data, Huang et al. (2010) is therefore considered to be the key study for the soil compartment.

3.1.2.5 Summary and discussion on biodegradation

Given the very low water solubility of decaBDE, biodegradation of the dissolved substance in water is not expected to be a significant removal pathway. This is confirmed by the results of the only ready biodegradation test available, which showed no mineralisation.

A wide range of information is available on biological transformation of decaBDE in other matrices:

- Canadian-government funded studies suggest the formation of small amounts of nona- and octaBDEs over 30 days in lake sediment, under environmental conditions that most closely resemble those of central/northern Europe. Until they are fully reported, it is not possible to draw firm conclusions at this stage.
- Other studies provide evidence that a range of sediment- and soil-dwelling micro-organisms are capable of transforming deca-, nona- and octaBDEs to at least hepta- and hexaBDEs (e.g. Qiu et al., 2011; Robrock et al., 2009; Lee and He, 2010; Deng et al., 2011). Although these studies are not necessarily representative of environmental conditions, they indicate that such organisms are capable of performing the transformation. It appears that transformation to lower molecular weight PBDEs is possible over time frames of a year (or more in some cases, e.g. Tokarz et al., 2008). The influence of factors such as sediment/soil loading, microbial adaptation and community structure, temperature, light and sediment/soil characteristics is not well understood due to a lack of data.
- OctaBDE congeners can be formed by sewage sludge micro-organisms over a period of about eight months under suitable conditions. Whilst these findings do not suggest that tetra- to heptaBDE congeners would be formed in significant amounts during wastewater treatment processes (since sludge residence times are usually too short, at around 20 days), they do provide some supporting evidence that the reaction might occur over longer timescales in the environment under appropriate conditions.
- In soils, an average loss of decaBDE of 20% (range 6-35%) was observed over a two-month period in a greenhouse experiment involving plants (Huang et al., 2010). For the species associated with the highest level of transformation, at least 122 µg/kg dw of tetra- to heptaBDEs were formed (i.e. 2.7% of the total measured PBDE

[42] Welsh et al. (2009) found that the congeners of a commercial pentaBDE product (DE-71) had a strong affinity with both sterile and non-sterile soil, with recovery of all congeners from soil by acetone extraction dropping significantly over an eight week ageing period. This general phenomenon was so dominant that varying soil characteristics (organic matter content, clay content, and pH) had no significant effect on PBDE recovery. When zucchini and radish plants were grown for 10 weeks in treated soil that had been aged for 8 weeks, recovery of congeners was up to five times higher than it had been prior to planting. This again implies that the plants play a role in enhancing bioavailability of PBDEs.

concentration present at the end of the test). It appears that soil microbes (including mycorrhizal fungi) associated with the plant roots may have an important role in this degradation (Wang et al., 2011a). A simulation study without plants was inconclusive as to the extent of degradation due to a high level of variability, and provided no information on possible degradation products. Soil is a heterogenous matrix, and the influence of sewage sludge application on degradation rate is unknown. The influence of temperature is also unknown. Whilst these are uncertainties, the reaction appears to be environmentally relevant, and can be performed by a range of soil micro-organisms.

3.1.3 Transformation in an aquatic mesocosm

As mentioned in Section 3.1.2.2, two freshwater lake mesocosm experiments have been conducted as part of a Canadian government-funded project. The studies are reported here in a separate section to other degradation studies because it is not clear what mechanisms are associated with the observations (abiotic, microbial and *in vivo* fish metabolism might all play a role). Field work began in 2007 and completed in October 2009. Although a formal manuscript containing full experimental details is not yet available, a summary of the experimental set up and main initial findings has been provided by Orihel et al. (2009) [ABST] and Muir (2011) [ABST], and oral or poster presentations have also been made for several scientific audiences (e.g. Orihel et al., 2010a [ABST] & 2010b [ABST]). Further details were provided directly by the researchers on request (Muir & Orihel, 2011; Orihel, 2011). Some or all of the experiments will be reported in the open scientific literature in due course and the interpretation of some of the findings might be subject to change. The available information is outlined below. (This work has not been summarised in previous EU risk assessment reports, and only partially in the registration dossiers.)

i) ***Experiment 1***: Four mesocosms were installed in the south end of Lake 240 (depth (z) = 2.6 metres) in August 2007. Each mesocosm was constructed from a rigid 10-metre diameter floating collar and a flexible cylindrical plastic wall that was secured to the lake bottom. The enclosures were open to the atmosphere and lake sediments. Strips of wall material were hung from the centre of each mesocosm for periphyton colonization. On 6 September 2007, three of the mesocosms received wet lake sediment fortified with a commercial decaBDE technical product (DE83R; Great Lakes Chemical Corp.; analysis showed that the nonaBDE content was below 1%), which was sprayed over the water surface as a dilute slurry of sediment particles. Each mesocosm received a different dose, resulting in a "low" (0.023 g), "medium" (0.21 g) and "high" (1.9 g) treatment. The fourth mesocosm ("control") did not receive any decaBDE.

   In October 2007, water, suspended particles, sediments[43], and biota samples were collected from the mesocosms. Strong winds during Spring 2008 dislodged the base of the mesocosms from the lake bottom. In May 2008, sediment cores and periphyton strips were collected from all mesocosms, and a few marked fish were captured from the "low" mesocosm. The experiment at this site was then terminated, and the mesocosms re-located (see Experiment 2 below). The centre of each mesocosm was marked with an underwater float, and an underwater fence (constructed from vapour barrier and wooden posts) was installed around the site of the "high" mesocosm. Surface sediments at the location of the original "high" mesocosm site were subsequently sampled twice a year until October 2009 (i.e.

[43] Three layers were sampled for each sediment core: top 0–1, 1–2, and 2–4 cm. The top 0–1 cm sample was analyzed for every core, and all three layers were analyzed for a subset of the cores. The graphs typically show data for the 0–1 cm layer.

around two years post-treatment). Details of the analytical method are provided for Experiment 2 below.

Eight months after the decaBDE addition, the sediments contained a range of lower PBDEs (Figure 5), which were either absent or at trace levels in the control.

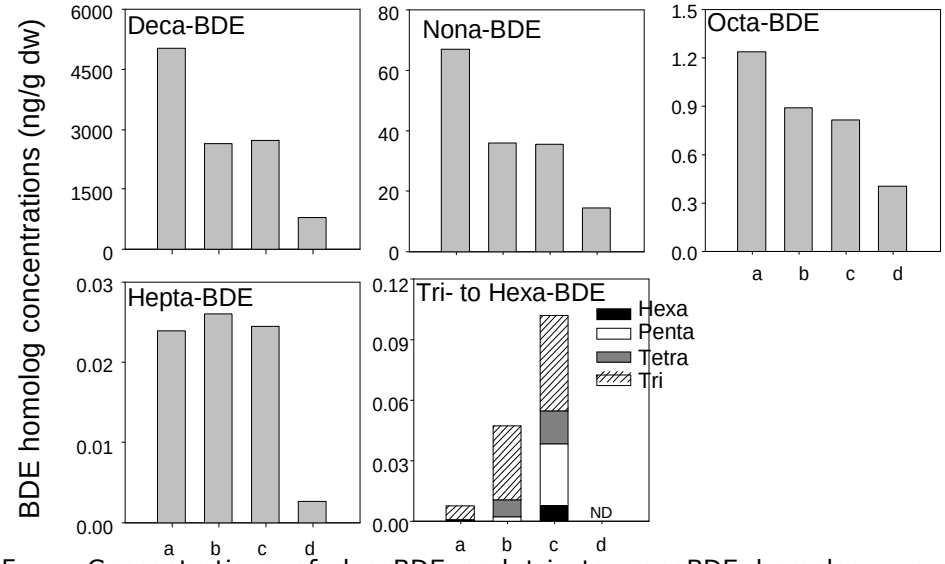


Figure 5: Concentrations of decaBDE and tri- to nonaBDE homologue groups in replicate sediment samples (a – d) from the "high" mesocosm in May 2008, i.e. 8 months post-treatment (Experiment 1)

The variable decaBDE concentration between replicates is due to the method of addition. The sediment slurry that was used for spiking was a profundal sediment of very fine texture. This was sprayed on to the surface of the mescosm, which had an area of 78.5 m^2, and so coverage would have inevitably been uneven. The sample cores had a cross-sectional area of 19.6 cm^2. Therefore it is not surprising that individual sediment samples were so variable. This variation does introduce a complication to the further analysis of the results, since a low decaBDE concentration was not necessarily representative of the intended treatment (it might have been more similar to the "medium" treatment, for example). Another consideration is that the reported PBDE levels relate to those congeners that were analysed. It is possible that some other congeners might have been present but not quantified.

Based on Figure 5, it can be roughly estimated that nona- and octaBDEs accounted for up to 2% and about 0.02-0.05%, respectively, of the total PBDE concentration by weight eight months' post-treatment. It should be noted that this included the winter period, when ice cover and low temperatures would be expected to have significantly curtailed any biotic or photolytic degradation.

One year after the addition, there had been no significant decline in decaBDE concentrations in surface sediments at the site of the "high" mesocosm (the highest concentration meausured was 5,500 ng/g dw; the background concentration in the lake sediment was about 3 ng/g dw). However, the percentage of decaBDE in the total PBDE pool in surface sediments decreased from 99% to 89% over this period, largely due to an increase in nonaBDEs (BDE-206, -207 and -208) during the summer months.

ii) ***Experiment 2***: The four mesocosms from Experiment 1 were relocated to the north-west corner of Lake 240 (z = 2.3 m) in June 2008. On 18 June 2008, the water surface of three of the mesocosms was sprayed with dilute slurry of freeze-dried sediment that had been fortified in the laboratory with the same commercial decaBDE technical product as Experiment 1. Each mesocosm received a different dose, resulting in a "low" (0.039 g), "medium" (0.28 g) and "high" (2.3 g) treatment[44]. The fourth mesocosm ("control") did not receive any decaBDE. The walls were re-used without decontamination since the plastic material showed little sign of periphyton growth and the same walls were used for the same dose in both experimental set ups (subsequent mass balance calculations have shown that the total mass of PBDE on the walls was negligible compared to the dose applied).

In early July 2008, the mesocosms were stocked with eggs of a common mayfly species (*Hexagenia* sp.) native to the lake (2,250 eggs per mesocosm). On 15 July 2008, Yellow Perch (*Perca flavescens*) aged one year or more (length 75 mm, weight 4 g) were captured from the lake, marked with dye, and 30 fish were stocked in each mesocosm. An initial sample of 16 fish was collected at the time of stocking. Strips of mesocosm wall material (10 cm x 1.5 m) were suspended in the mesocosms for periphyton colonization. The mesocosms were also naturally colonized with zooplankton and benthic invertebrates (Ephemeroptera).

Intact sediment cores (four per mesocosm), water and suspended particles (~100 litre samples collected using XAD-2 resin columns with 1 µm glass fibre cartridge filters using a battery operated pumping system), strips of wall material and zooplankton were sampled from the mesocosms in July and October 2008 (i.e. one and four months post-treatment). Sediment cores were extruded into 0-1 and 1-2 cm slices and pooled. Zooplankton was collected with a 170 µm sweep net and composite, freeze-dried samples were analyzed. An attempt was made to catch Yellow Perch (10 per mesocosm) with minnow traps and gill nets in October of each year. Freeze-dried whole bodies minus guts, jaw and liver[45] were analyzed for PBDE concentrations. Sampling of biota generally occurred prior to sediment sampling. Samples were stored at -20°C prior to analysis.

Like Experiment 1, the mesocosms were found to be dislodged during Spring 2009. They were carefully repositioned over their original sites. Further samples were collected in May 2009 (eleven months after initial treatment). However, the "low" and "medium" mesocosms were re-treated with decaBDE on 11 June 2009 (with 2.27 g and 0.25 g, respectively) using freeze-dried sediment as before. These were subsequently termed the "low/high" and "medium/medium" mesocosms. Further zooplankton, periphyton and sediment samples were collected in July 2009 following re-treatment with decaBDE (one month after re-treatment), and then in October 2009 (when fish were also sampled) (i.e. sixteen months post-treatment for the "high" mesocosm, and four months following re-treatment in the other two mesocosms).

Frozen samples were stored in bags and kept in the dark prior to analysis (in some cases the time between collection and analysis was a year or so). Wet sediment samples were mixed with a drying agent, spiked with BDE-71 and ^{13}C-decaBDE and extracted using pressurized fluid extraction (Dionex Accelerated Solvent Extraction

[44] These application rates were designed to represent 0.1, 1 and 10 times the concentration of 240 ng/g dw that had previously been measured in Lake Ontario sediments.

[45] Fish jaws and livers were analysed separately. Given the small sample weight, livers were pooled for a given mesocosm and time point, and the results have not yet been reported. Jaws were removed for thyroid analysis.

(ASE)) using dichloromethane. Water filters were ASE extracted using dichloromethane. Wall strips were extracted by shaking with dichloromethane, and the solvent extracts were exchanged into hexane and then fractionated on an activated silica column using hexane and (1:1) hexane: dichloromethane. Extracts were reduced in volume and taken up in isooctane for gas chromatographic (GC/MS) analysis. XAD resin columns, pre-spiked with BDE-71 and ^{13}C-decaBDE, were eluted with methanol and dichloromethane; solvent extracts were combined and washed with 3% sodium chloride and the dichloromethane dried on sodium sulphate, before fractionation as above.

Extracts were screened for 45 individual PBDEs (including all octa- and nonaBDEs) by GC-electron capture negative ion MS (GC-ECNI/MS) using a DB-1MS capillary column (15 or 30 m, 0.32 mm, 0.10 µm). Samples were analyzed on an Agilent 6890 GC coupled to a 5975 MS. All PBDEs were monitored at m/z 79/81 and quantified using external standard calibration. Values below instrument detection limits (S/N=3) were assigned zero. Laboratory blanks consisting of all materials (filters, XAD) were analysed with each batch. Samples from the control mesocosm served as additional controls. All results were blank corrected. Blank contamination was not significant in sediments or zooplankton. Fish samples contained decaBDE concentrations that were not clearly linked to the dose that was added, which might indicate a degree of contamination. However, the lower congener profiles in fish are correlated with the dosing regime, and are considered reliable. Limits of detection were based on the standard deviation from two blank samples.

Full results are not yet available (in particular, the October 2008 dataset has only partially been reported, and some of the sediment and periphyton samples collected in 2009 have not been analysed yet). The following information is publicly available.

A dose-dependent gradient was produced among the three treated mesocosms. One month after the initial application, average concentrations of decaBDE in surface sediments (top 1 cm layer) were 13, 216 and 998 ng/g dw in the "low", "medium", and "high" mesocosm, respectively. The "low" mesocosm concentration was not significantly higher than the control mesocosm (3 – 6 ng/g dw, which was similar to other remote lakes in the region). It was therefore decided to re-dose this mesocosm in June 2009 to replicate the original "high" dose.

DecaBDE transformation products were observed in surface sediments as early as one month after test substance addition in all treatments. The major products were two nonaBDEs (BDE-206 and -207). In the "high" mesocosm, these two congeners were present at an average total concentration of about 550 ng/g dw after 12 months (and were virtually undetected in the control) (values read from a graph). OctaBDEs (BDE-197, -195, -196, -200 and -198/199/203) were minor products at one and eight months. In the "high" mesocosm, the total concentration of these congeners was about 0.8 ng/g dw after eight months (values read from a graph). Three specific tri-, tetra- and pentaBDE congeners were also observed in the "medium" and "high" mesocosms at total concentrations below about 0.25 ng/g dw (values read from a graph), whereas concentrations were near or at detection limits in the controls.

The percentage of decaBDE in the sum of total PBDEs was ~96% after both one and four months. The production of penta-, hexa-, hepta- and octaBDEs was roughly ten times higher in Experiment 2 than 1 while the percentage of nonaBDEs was similar in both experiments. The congener pattern was also similar in both experiments, and BDE-205 and BDE-194 (both octaBDEs) were not detected. The predominance of BDE-206 and -207 and BDE-196, -197, -200 and -201 suggested progressive loss of bromine from positions 5- and 6- on the aryl rings.

When the suspended wall material samples were analysed, decaBDE was only detected in the "medium" and "high" mesocosms. The overall mass adsorbed to the walls was low, i.e. ~0.4 mg in the "high" mesocosm after one month as decaBDE. NonaBDEs were detected in the "high" mesocosm after one month, but not after four months. In contrast, hexa- to octaBDEs were not detected after one month, but were found after four months in both the "medium" and "high" mesocosms. The plastic wall material may have acted as a passive water sampler. For example, controls also had detectable levels of BDE-28, -47 and -99, and the congener pattern resembled the dissolved phase.

DecaBDE was detectable in filtered water at concentrations of 5-19 ng/l from one month to 12 months post-treatment ("high" dose). The three nonaBDEs (BDE-206, -207 and -208) were also consistently present in filtered water. Tri- and tetraBDEs were the predominant minor products at one month and 9 months post-treatment but were not detected at 12 months.

DecaBDE along with the three nonaBDEs were present in suspended particles (diameter above 1 µm). The pattern of tri- to octaBDEs in suspended particles was dominated by octaBDEs. Higher concentrations were found at 12 months compared to 9 months.

Zooplankton in the "high" mesocosm contained tetra- through to nonaBDEs in higher amounts than the control after the first year. The PBDE concentrations were correlated with the decaBDE dose added to the mesocosms. The "low" mesocosm contained higher amounts of tri-, tetra- and pentaBDEs than the "high" mesocosm. In the second year, the highest concentration of PBDEs was in zooplankton in the "low/high" mesocosm, which received a high dose in the second year. A significant amount of PBDE was also present in the "high" mesocosm that had not received any dose in the second year. This demonstrates that decaBDE's degradation products continued to be available to biota in the water column for at least a year after deposition in the lake.

Yellow Perch captured from the treated mesocosms in October 2008 (three months post-treatment) had higher carcass concentrations of decaBDE on average than fish captured from the control mesocosm, although there was a high degree of variation among individuals (Figure 6). Although the aim was to capture ten fish per treatment at each time point, this was not always possible. The reported results are for all fish that were captured.

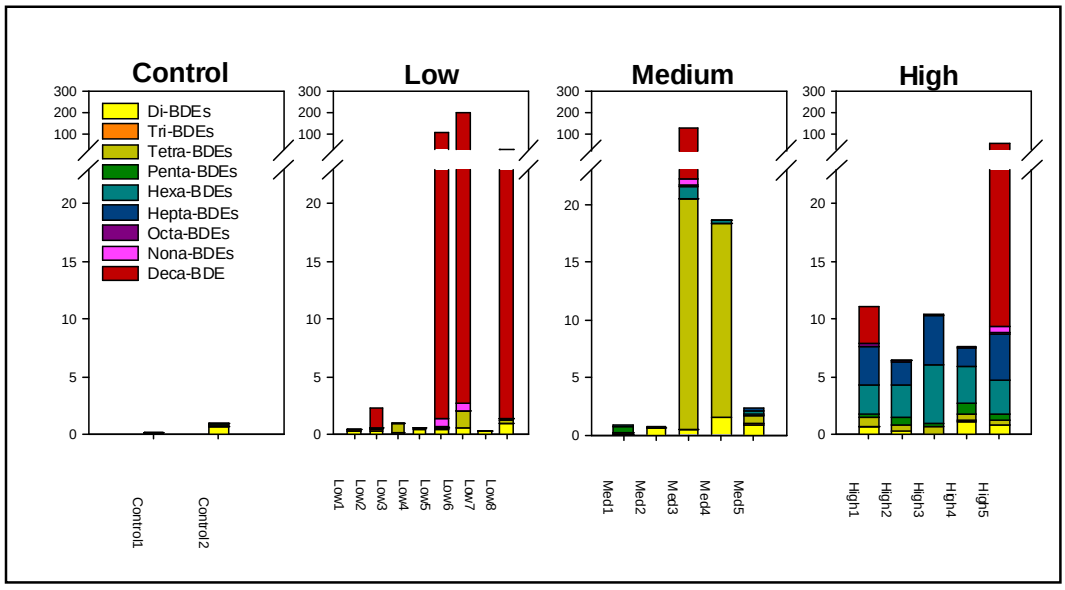


Figure 6: Concentrations of PBDE homologue groups in individual yellow perch carcasses from the control, "low", "medium" and "high" mesocosm, captured in October 2008 (units are ng/g dw)

PBDE concentrations in control fish were below the detection limit except for tri- and tetraBDEs, which were present at concentrations below about 1 ng/g dw. Fish from the "medium" and "high" mesocosms had higher concentrations of tetra- to nonaBDEs than both the control and "low" mesocosms. For the "high" mesocosm, addition of 2.3 g of decaBDE to a water volume of 180 m^3 resulted in fish concentrations of hepta- and hexaBDEs around 5-10 ng/g dw in total after three months. In contrast, tetraBDEs seemed to be more prevalent in the "medium" mesocosm. This observation suggests that either fish were accumulating lower PBDE congeners from their environment, or taking up decaBDE and debrominating this compound within their bodies (or a combination of both).

Fish that were captured from the mesocosms in October 2009 (three months after the 2009 re-treatment with decaBDE) also showed evidence of lower PBDE congener accumulation and/or decaBDE debromination. Fish from the "low/high" (one fish) and "medium/medium" mesocosms had higher concentrations of tetra- to octaBDEs than fish from both the control mesocosm and a reference lake. This was also true of fish from the "high" mesocosm that was treated with decaBDE in 2008 but not 2009, demonstrating that decaBDE (or its breakdown products) remains bioavailable to aquatic biota for at least one year after deposition to lake sediments. The lower PBDE congener pattern and amounts in the single fish collected from the "low/high" mesocosm were similar to those in the "high" mesocosm sampled a year before.

Orihel et al. (2009) [ABST] mentioned that BDE-126 was detected in some fish collected in 2009 from the "medium" and "high" mesocosms, but not from the control or "low" mesocosms. As noted in Section 3.1.2.2, this pentaBDE congener is a marker for potential abiotic degradation, and its presence suggests that other intermediate PBDE congeners of concern would also be present.

Some additional analyses are planned to start in September 2011 on both the biota and sediment samples with the goal of providing data to fully interpret the observed bioaccumulation of lower molecular weight PBDEs and estimate a mass balance in the mesocosms. The current picture of congener distribution in three matrices is presented in Figure 7.

As can be seen, heptaBDEs accounted for a few per cent of the PBDE profile in treated sediments. In fish, the contribution of hexa- and heptaBDEs was much more prominent than in controls, at least in the "high" mesocosm. The researchers intend to calculate the overall level of decaBDE degradation and percentage w/w formation of each of the lower PBDE congener groups in the mesocosms over the duration of the experiment, but have not done this yet.

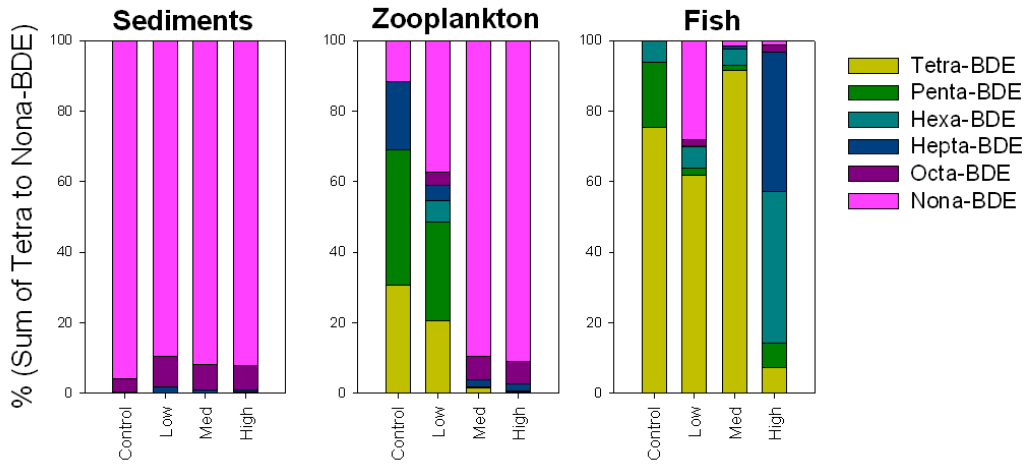


Figure 7: Distribution of tetra- to nonaBDE homologue groups in sediment, zooplankton and fish from the control, "low", "medium" and "high" mesocosm in the first year after treatment

Discussion

The registration dossiers only consider some of the early data summaries for this study. They conclude that it did not adequately control for variables affecting results (such as perturbation due to storms, atmospheric input and wave redistribution), and that the conclusions drawn by the authors do not follow from the reported results.

This study has not been fully reported yet. Whilst a reliability marking cannot be assigned to the study for this reason, the findings are the first to provide direct evidence of transformation of decaBDE in the aquatic environment under natural field conditions. As such, it is important, and so it is considered relevant to include the available data in this dossier at this stage (if further details emerge during the public consultation period, they can be added in due course).

The incomplete reporting of the results, number of congeners investigated (limited by the availability of reference standards), changing exposure conditions over the course of the experiments, high degree of variation between replicates and differences in observed PBDE concentrations between different treatments make interpretation difficult at this stage. This is the nature of most field-based studies: the high degree of realism comes at a cost of high variability and lack of control over conditions. However, the absence of

significant inputs from other PBDE sources (i.e. commercial penta- and octaBDE products) means that the findings can be related to the presence of decaBDE[46].

It is clear that decaBDE transformation products (tetra- through to nonaBDEs) accumulated in sediments over time, with concentrations increasing up to ten-fold over 3 to 5 months. Initial debromination occurred within a time frame of weeks, via progressive loss of bromine from positions 5- and 6- on the aryl rings, primarily yielding 2,2',3,3',4,5,6- substituted nonaBDEs, with smaller amounts of tri- to octaBDEs. HeptaBDEs were formed within months in the "high" and "medium" treatments, which had a decaBDE concentration lower than those found at contaminated sites[47]. Differences in the proportion of debromination products between Experiment 1 and 2 could be due to the time of year (Experiment 1 started in colder conditions (September-October) whereas Experiment 2 started in mid-summer). Degradation appears to occur more rapidly over the summer period, when penta- and hexaBDEs were observed one month after decaBDE addition. Furthermore, these debromination products were also detected in fish indicating that they are either bioavailable or formed following ingestion and transformation of decaBDE by the fish (or a combination of both routes). The congener patterns of octa- and nonaBDEs in both mesocosm experiments were similar to those observed in natural lake sediments in Switzerland by Kohler et al. (2008).

Fish that were present in the treatments had accumulated total hepta-/hexaBDEs concentrations up to around 5-10 ng/g (µg/kg) dw three months after addition of the decaBDE dose. It should be noted that the fish livers were analyzed separately. Since decaBDE is often associated with liver tissue (e.g. Stapleton et al., 2006 and Kuo et al., 2010b (see Section 3.3.1.2)), the measured tissue concentrations for fish carcass alone might have under-estimated the actual concentrations that were present in the whole fish.

The experiment provides an indication of PBDE formation following application of a single dose. The highest nominal loading was lower than that encountered in polluted sediments in Europe. The studies were not intended to provide information on the level of PBDE formation that could arise if deposition was continuous. It might be expected that PBDEs levels would be higher under such circumstances. The mesocosms were positioned in only one region of the lake (i.e. shallow littoral – oxic, light, warm), so the influence of other conditions could not be examined.

The relevance of the findings at this relatively pristine Canadian site for European conditions needs to be considered. Mean air temperatures at this location are typically *minus* 17°C in January and 19°C in July, and there is extensive snow and ice cover over the winter period. These conditions are most comparable to those found in northerly latitudes (e.g. Scandinavia and some of the Baltic states). The sunlight intensity (based on latitude) would be similar to that experienced by central Europe. The influence of warmer temperatures and/or greater sunlight exposure (to be found in more southerly locations) is unknown. However, since transformation appeared to proceed more quickly during the summer months, it might be expected that these factors would lead to greater degree of transformation than was observed in these studies.

[46] This locality has a low level of anthropogenic activity, but decaBDE and some lower PBDE congeners were already present in the lake at low concentrations. However, this background contamination is not considered likely to influence the interpretation of the results.

[47] The "high" mesocosm had a decaBDE concentration of 998 ng/g (µg/kg) dw after one month. The highest reported sediment concentration cited in the European risk assessment reports was 12,500 µg/kg dw for a Spanish river close to sources of release (Eljarrat et al., 2007). It is possible that levels could be higher at other locations, since the number of sampling sites is limited.

3.1.4 Summary and discussion on degradation

Abiotic degradation

DecaBDE may reside in the atmosphere on fine particulates for days during dry periods. Phototransformation to several per cent w/w nonaBDEs can be expected under such conditions. These will ultimately be deposited to sediments and soils. Small amounts of other substances such as octa- and heptaBDEs and brominated dibenzofurans might also be formed in some circumstances, although it is not possible to assess the likely extent of this.

In aquatic environments, decaBDE has the potential to photodegrade relatively quickly, and nona-, octa-, hepta- and hexaBDE congeners have been observed to be formed in freshly spiked sediment following exposure to light over 96 hours under laboratory conditions. Other substances might also be formed, including brominated dibenzofurans. In practice, only a very small fraction of the total decaBDE present in aquatic environments will be available for photodegradation (due to light attenuation, shielding, etc.). A recent *in situ* sediment degradation study did not reveal any significant influence of light on the observed degradation, although this test was of relatively short duration (12 days). Ageing might also play a role in reducing the potential for this reaction with time. Photolysis in sediments might therefore not be an important mechanism in the environment. A similar conclusion can be drawn for soil.

Reaction with reductants (e.g. iron-bearing minerals and sulphide ions, etc., some of which may be water-soluble) present in anaerobic conditions in both sediments and soils is a possible additional abiotic transformation route, but it is not possible to estimate the extent or rate of any transformation based on the available data.

Biotic degradation

Degradation under environmentally realistic conditions in sediments and in aerobic soil in the presence of plants have both been shown to lead to the formation of tetra- to heptaBDE congeners (as well as octa- and nonaBDEs). The key data are provided by a mesocosm experiment in a Canadian lake (Orihel et al. (2009) [ABST] and Muir (2011) [ABST]) and the soil experiments of Huang et al. (2010) and Wang et al. (2011a). These provide strong evidence that hexa- and heptaBDE congeners can be formed under either actual or realistic worst case environmental conditions in sediments and soils.

These findings are supported by a range of other laboratory studies (e.g. Huang et al., 2011), and also monitoring data, although various factors mean that these other studies only provide equivocal evidence of transformation (for example, several studies only measured a few possible congeners; most experiments are of relatively short duration; and the chosen test conditions are often difficult to extrapolate to the environment). The presence of many congeners in samples and laboratory blanks due to previous releases from other commercial PBDE products is an additional complication. It is also possible that as sediments and soils age, any decaBDE remaining might become less available to micro-organisms (reducing the rate of transformation but also increasing overall persistence).

3.2 Environmental distribution

3.2.1 Adsorption/desorption

The registration dossiers have two study summaries for this end point based on quantitative structure-activity relationship (QSAR) calculations, without any indication of the reliability of the methods for this substance or analysis of sensitivity to the input parameters (PCKOC v.1.66 and EPIwin Level III Fugacity Model). Nevertheless, the results are broadly consistent with EC (2002), which estimated an organic carbon-water partition coefficients (K_{oc}) for decaBDE in the range 150,900 to 149,000,000 l/kg (estimated from a log K_{ow} of 6.27 and 9.97, respectively).

Watanabe (1988) measured a sediment-water partition coefficient for decaBDE, by adding sediment, to which the substance was already adsorbed, to clean water. A Kp(sed) of 79,433 l/kg was obtained. No information on the organic carbon content of the sediment was reported. If it is assumed that the sediment is 5% organic carbon, then values of the partition coefficient for soil and suspended sediment can be estimated as Kp(soil) = 31,773 l/kg and Kp(susp) = 158,866 l/kg, assuming organic carbon contents of 2% and 10% for soil and suspended sediment respectively (i.e. K_{oc} = 1.59 x 10^6 l/kg, which is in good agreement with the estimates given above).

DecaBDE is therefore expected to adsorb strongly to organic matter in suspended particles, sewage sludge, sediment and soil. Given its low water solubility (<0.1 µg/l), mobility in soils is also likely to be of low.

3.2.2 Volatilisation

The registration dossiers have one study summary for this end point based on QSAR calculations, without any indication of the reliability of the method for this substance or analysis of sensitivity to the input parameters (HENRY v.3.10, within EPIwin v.3.04). The conclusion in the registration dossier is that decaBDE is not expected to volatilise from water into air.

EC (2002) concluded that the decaBDE's low vapour pressure (4.62×10^{-6} Pa at 21°C) means it is unlikely to volatilise readily from spillage to land. Although a Henry's Law constant of >44 Pa m^3/mol at around 20°C can be estimated from the water solubility and vapour pressure, this value is highly uncertain given the measurement difficulties for these two parameters. In practice, volatilisation from surface water and sewage works might occur to a small extent, but adsorption to suspended matter is likely to reduce this tendency significantly.

Once in the atmosphere, studies show that decaBDE is transported on air borne particles, which are susceptible to wet and dry deposition (e.g. ter Schure et al., 2004; ter Schure and Larsson, 2002). Further transport depends on the fate of the deposited particles, which may be governed by the level of wind erosion on land, and currents and surface layering in water.

Long-range transport in the air is considered further in Section 3.2.3.2.

3.2.3 Distribution modelling

3.2.3.1 Fugacity modelling

EC (2002) estimated that the overall removal of decaBDE during wastewater treatment would be 91.7% (91.4% resulting from adsorption to sewage sludge and 0.3% resulting from volatilisation to air) using the SIMPLETREAT model.

DecaBDE also has a high potential for adsorption to organic matter in sediments and soils, as indicated by a level III fugacity model (EQC V1.01) (Table 11).

Table 11: Level III fugacity modelling for decaBDE

Release[a]	**Predicted environmental distribution**			
	Air	**Water**	**Sediment**	**Soil**
Equal emission to air, water and soil	1.7×10^{-8} %	0.011%	0.70%	99.3%
100% emission to air	0.024%	6.0×10^{-3}%	0.38%	99.6%
100% emission to water	4.9×10^{-7}%	1.54%	98.5%	2.0×10^{-3}%
100% emission to soil	5.7×10^{-9}%	3.8×10^{-3}%	0.24%	99.8%

Note: a – All calculations were based on a release of 1,000 kg/hour to each compartment. The input parameters were a vapour pressure of 4.63×10^{-6} Pa (at 21°C), water solubility of 0.1 µg/l (at 25°C), log K_{ow} of 6.27, half-life in air of 94 days and infinite half-life in surface water, sediment and soil. A higher log K_{ow} value will make little difference to the overall distribution.

Consequently, sediments and soils are the primary compartments in which the substance will reside at steady state following release, and these are the most important in terms of the relevance of transformation.

3.2.3.2 Long-range transport potential

Several modelling studies of the long-range atmospheric transport potential of decaBDE have been performed (e.g. Wania and Dugani, 2003; Scheringer, 2009; Schenker et al., 2008a [ABST] and 2008b). The relevance of the models for decaBDE depends on uncertainties in the input data, as well as the way in which they take account of the transport of aerosols and atmospheric particulates, possible phototransformation, etc. For example, rain-out is an important removal mechanism for atmospheric particulates, and some models assume a constant particulate wash-out rate, and a single particle size (ECB, 2004). Particles with diameters of around 300 µm would be expected to deposit fairly quickly (in the order of minutes at typical wind speeds), whereas finer particles (with a diameter around a few micrometres) might remain airborne for hours or days, provided that they are not removed by wet deposition. Transport of fine particles over longer distances (1,000 kilometres or more) can be expected during periods of dry weather. Therefore, although a limited transport potential is often predicted because of fast deposition with aerosol particles, this should be considered with caution.

DecaBDE has been shown to have a widespread occurrence in the environment, particularly in sediments and sludges, but also biota and air. The available monitoring database is extensive and has been discussed previously (see EC (2002), ECB (2004 and 2007a) and EA (2009)). A number of studies have investigated the occurrence of decaBDE in remote regions and these findings are summarised briefly below.

Sediment and sewage sludge

- The levels of decaBDE in sediment cores from lakes along a north-south transect from southern Ontario and upper New York State to Ellesmere Island, Canada, were

studied by Muir et al. (2003) [ABST] and also reported by Breivik et al. (2006). With the exception of Lake Ontario, the lakes were all uninhabited or had a history of very little human disturbance. DecaBDE was found to be present in the most recent sediment layers from six out of the eight lakes sampled, but at very low levels (close to or below the detection limit) in samples collected north of 55°N. The sediment core data also appeared to show that the concentration was highest in the most recent layers. Concentrations were generally lower and the date of first occurrence was later in the more northerly samples.

- Hale et al. (2008) determined the levels of decaBDE (and nonaBDEs) in samples of waste water sludge from two research bases in the Antarctic. The levels were 1,320 µg/kg dry weight in a sample from the McMurdo research base and 219 µg/kg dry weight in a sample from the Scott research base. DecaBDE was also present in sediment at the outfall of the waste water treatment plant at the McMurdo research base at a concentration of 3,540 µg/kg organic carbon and the concentrations in sediment were found to decrease with increasing distance from the base. The detection of high levels of decaBDE in aqueous waste streams at Antarctic research stations shows that point sources may be significant even in supposedly remote regions.

Air and dust

- Su et al. (2007a [ABST] and 2007b) reported the results of the Arctic Monitoring and Assessment Programme (AMAP). Monitoring for PBDEs at Alert in Canada began in 2002 with the aim of establishing long-term trend data in concentration in Arctic air. DecaBDE was detected frequently in air. The average concentration found in the samples over a two year period was 1.6 pg/m^3 (range 0.091 to 9.8 pg/m^3). The authors also estimated the inter-annual time trend in the concentrations and determined that the levels of decaBDE found increased over the time period, with a doubling time around 6 years.

- Cheng et al. (2007) reported levels of decaBDE in air from the Waliguan Baseline Observatory in northwestern China. The observatory is part of the Global Atmospheric Watch network of the World Meteorological Organization and is located on the edge of the northeastern Tibetan Plateau at a height of 3,816 m above sea level. Air samples (both particulate and gaseous) were collected. The levels of total PBDEs measured at the site were in the range 2.2 to 15 pg/m^3, with a mean of 8.3 pg/m^3. DecaBDE was reported to be the third most predominant congener in the samples (the actual levels of decaBDE found were not given).

- Meyer et al. (2012) analysed snow cores from the Devon Ice Cap in Nunavut, Canada, which correlated with the period from approximately 1993 to 2008 (see Section **Error! Reference source not found.**). Deposition fluxes of decaBDE showed no clear temporal trend, and ranged between 90 and 2,000 pg/cm^2/year.

- Hale et al. (2008) determined the levels of decaBDE (and nonaBDEs) in samples of indoor dust from two research bases in the Antarctic. The levels of decaBDE found in the dust samples were 4,160 µg/kg in a sample from the McMurdo research base and 1,650 µg/kg in a sample from the Scott research base. NonaBDEs were also present in the samples, with the concentration of BDE-206, BDE-207 and BDE-208 being 201, 510 and 163 µg/kg respectively in the sample from the McMurdo research base and 56, 69 and 43 µg/kg respectively from the Scott research base.

Biota

- SFT (2002) determined the concentrations of decaBDE in samples of moss (*Hylocomium splendens*) from eleven locations across Norway. DecaBDE was found to be present in every sample with concentrations in the range 0.025-0.66 µg/kg wet

weight. The report indicated that the presence in moss was indicative of (particulate) transport of the substance via the atmosphere.

- Gabrielsen et al. (2005) collected fifteen liver samples from two breeding colonies of Northern Fulmar (*Fulmarus glacialis*) on Bjørnøya (Svalbard) in the Norwegian Arctic during June-July 2003 (six females and nine males). DecaBDE was detected in one of the fifteen samples at a concentration of 206 µg/kg wet weight (the detection limit of the method was not given).

- Knudsen et al. (2005) found decaBDE to be present in eggs from a number of species of seabirds from northern Norway and Svalbard. In a follow-up study, Knudsen et al. (2007) collected liver and brain tissue samples from twenty-one Glaucous Gulls (*Larus hyperboreus*) and two Great Black-backed Gulls (*Larus marinus*) found dead or dying on Bjørnøya during 2003, 2004 and 2005. DecaBDE was detected in five of the Glaucous Gull brain tissue samples (24%) at a mean concentration of 2.9 µg/kg lipid (the maximum concentration was 9.5 µg/kg lipid). It was detected in all but one Glaucous Gull liver sample (95%) at a mean concentration of 186 µg/kg lipid (the maximum concentration was 2,586 µg/kg lipid). The report does not present detailed data for *L. marinus*.

- Verreault et al. (2004, 2005 and 2007) also reported decaBDE concentrations in samples of Glaucous Gull (*L. hyperboreus*) eggs and/or blood collected from Bjørnøya. The substance was found to be present in some samples at low concentrations. For example, decaBDE was detected in six out of twelve blood samples collected from males during May-June 2004, at concentrations up to 0.21 µg/kg wet weight (similar results were obtained for females). In contrast, decaBDE was "virtually non-detectable" in samples of plasma and egg yolk collected in May and June 2006.

- The levels of decaBDE in Ringed Seals (*Phoca hispida*) from the Holman Islands in the Canadian Arctic were studied by Ikonomou et al. (2000 [ABST] and 2002). The detector response for decaBDE from the samples was the same as that for the procedural blanks (162-236 ng/kg), indicating that little or no decaBDE was detected in the samples.

- Gabrielsen et al. (2004) analysed adipose tissue from Polar Bears (*Ursus maritimus*) for the presence of decaBDE. The samples were collected from fifteen individuals from Svalbard, Norway in April 2002. DecaBDE was not detected in any of the samples analysed, at a detection limit of 0.1 µg/kg wet weight. Verreault et al. (2005) collected blood samples from fifteen adult female polar bears during April 2002 in Svalbard (presumably the same individuals as in the previous study). DecaBDE was detected in one bear's blood at a concentration of 0.1 µg/kg wet weight. The detection limit of the analytical method was 0.06 µg/kg wet weight.

- The levels of decaBDE in invertebrates (an ice-associated omnivorous amphipod (*Gammarus wilkitzkii*)), Polar Cod (*Boreogadus saida*), Ringed Seals (*Pusa hispida*) and Polar Bears (*Ursus maritimus*) from Svalbard, Norway have been determined by Sørmo et al. (2006a [ABST] and 2006b). DecaBDE was found at the following concentrations:

  - *amphipod*: 0.28 µg/kg whole body weight (mean) (~7.2 µg/kg lipid);
  - *polar cod*: 0.020 µg/kg whole body weight (mean) (~0.20 µg/kg lipid)
  - *ringed seal (blubber)*: 0.006 µg/kg whole body weight (~0.02 µg/kg lipid), one of six samples only
  - *polar bear*: 0.022 µg/kg whole body weight (mean) (~0.09 µg/kg lipid in adipose).

Discussion

DecaBDE is associated mainly with particulates in the atmosphere and some modelling studies suggest that it will have a limited potential for long-range atmospheric transport because of rapid removal during wet deposition. However, it could be transported over longer distances during dry periods. The available monitoring data show that decaBDE is found in remote regions at low concentrations in air, sediment and wildlife. Local sources might be involved in some cases (for example decaBDE has been found in aqueous waste streams from Antarctic research stations). Migratory wildlife might also be exposed on their wintering grounds. Nevertheless, occurrence in lake sediment cores far from human habitation and detection in the air at remote locations suggests that long-range transport is occurring to some extent.

3.3 Bioaccumulation

Bioaccumulation data for decaBDE itself are not directly relevant to this dossier, because of its focus on biotransformation. The registration dossiers have six key (and one supporting) study summaries for aquatic/sediment bioaccumulation based on nine references (Stapleton et al., 2004a & 2006; Thomas et al., 2005; Tomy et al., 2004; CITI, 1992 (plus an unspecified publication); Kierkegaard et al., 1999; and Kuo et al., 2010a[48]). The CITI (1992) study was summarised in EC (2002) and the registrants consider that it provides a valid fish bioconcentration factor (BCF). However, several methodological deficiencies (summarised in ECB, 2007a) mean that it is not in fact valid, so it is not relevant to consider it further. ECB (2007a) concluded that the available evidence suggests that the fish BCF is below 2,000 l/kg.

Kuo et al. (2010a) investigated the biomagnification of decaBDE in the Lake Michigan food web. (This study was not summarised in previous EU risk assessment reports.) Plankton, *Diporeia*, Lake Whitefish, Lake Trout and Chinook Salmon were collected from Lake Michigan between April and August 2006. Fish liver and muscle and whole invertebrates were analyzed, and carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) were also quantified to establish the trophic structure of the food web. DecaBDE concentrations ranged from 0.184 to 1.23 µg/g on a lipid weight basis in all three fish species. A higher concentration (144 µg/g lipid) was detected in *Diporeia*, and this was thought to be one of the main dietary sources of decaBDE for fish in the lake. Based on analysis of $\delta^{15}N$ and decaBDE concentrations, as well as calculated biomagnification factors (BMFs) for different feeding relationships, decaBDE did not biomagnify. A significant negative correlation between decaBDE and trophic level was found in this food web. The decreasing concentration of decaBDE at higher trophic levels was thought to reflect partial uptake and/or biotransformation. This finding is consistent with several other studies summarised in EA (2009) but not included in the registration dossiers[49]. The registrant does not assign a validity mark to this study.

All the other studies considered by the registrants are summarised in Section 3.3.1.2, together with additional information (the Thomas et al. (2005) reference is not explicitly discussed because it does not address metabolism). Some limited uptake was seen in

[48] One further reference concerns a 48-hour fish study, which is not considered relevant for the purposes of this dossier. The registrants also include two summaries for earthworm accumulation, which are not considered further.

[49] Law et al. (2006) reported biomagnification of decaBDE in a Lake Winnipeg food web, based on both BMF and trophic magnification factor measurements. However, a number of drawbacks in the study methodology were identified in ECB (2007a). The conclusion of that evaluation was that whilst the study provided some evidence for increasing concentrations of decaBDE with trophic level within this food chain, the major uncertainties in the data meant that it was not possible to conclude definitively that biomagnification was occurring.

experiments with fish exposed via food in a non-standard guideline study over periods up to 120 days, but the tissue concentrations were much lower than those present in the food.

DecaBDE has been detected in the tissues of a large number of species – including zooplankton, fish, and both aquatic and terrestrial invertebrates, birds and mammals – in many geographical locations, as described in EC (2002), ECB (2004 and 2007a), EA (2009), and Environment Canada (2010). Analysis for decaBDE requires a number of precautions, which introduces some uncertainties for some studies (particularly older ones). It is difficult to establish median concentrations in different aquatic biota given that the substance is frequently not detected (i.e. <<1 µg/kg wet weight). When it is detected in fish (often associated with areas that are known to be contaminated by local sources), maximum concentrations are around a few micrograms per kilogram wet weight (ww) in general. Although sample numbers tend to be small, several studies have detected decaBDE in a variety of species at the top of food chains, including predatory birds such as Peregrine Falcon (*Falco peregrinus*) (including eggs), mammals such as Red Fox (*Vulpes vulpes*) and aquatic organisms such as Bull Shark (*Carcharhinus leucas*) (e.g. Lindberg et al., 2004; Voorspoels et al., 2006; Johnson-Restrepo et al., 2005). In general terms, terrestrial species appear to have higher levels than aquatic ones (e.g. Jaspers et al., 2006). In some samples, decaBDE can be the predominant PBDE congener present. Concentrations are typically in the range 1-100 µg/kg ww, with a maximum detected concentration of about 420 µg/kg ww (Chen & Hale, 2010). In humans, the substance has been detected in blood serum samples in the range of 9.1 to 33.9 µg/kg lipid (with a maximum concentration of 240 µg/kg lipid), although it is frequently not detected. In one study of breast milk, it was quantified in around half of 128 samples at a mean concentration of 0.21 µg/kg lipid (the maximum concentration was 4.5 µg/kg lipid).

Uptake from ingestion therefore seems to be important, and at least some food chains and species appear to accumulate the substance to a greater extent than expected from the laboratory fish and rodent data alone. For example, it is rapidly and extensively absorbed from food by Grey Seals (*Halichoerus grypus*) (Thomas et al., 2005).

Environment Canada (2010) performed a weight of evidence analysis using a wide range of data that had been published up to and including 2009. They concluded that "most available data show that decaBDE has limited potential to bioaccumulate or biomagnify in the environment", but pointed out that "the substance is increasing in concentrations in some wildlife species, and some data suggest that it has reached concentrations in some organisms interpreted to be 'high'." Some recent studies suggest that biomagnification is possible in terrestrial food chains (e.g. Yu et al., 2011, summarised in Appendix 1), although the reported levels in terrestrial top predators are lower than those typically found for very bioaccumulative substances such as pentabromodiphenyl ether and hexabromocyclododecane (see ECB, 2007a for a comparison).

3.3.1 Transformation in aquatic species

The focus of this section is the biotransformation of decaBDE to lower PBDEs by fish[50].

[50] Few data are available for invertebrates. Riva et al. (2007) exposed zebra mussels (Dreissena polymorpha) to decaBDE (purity: 98%; the major impurities were two nonaBDE congeners) in water at nominal concentrations of 0.1, 2 and 10 µg/l under a daily renewal regime for up to 168 hours. The tanks were screened against direct sunlight to avoid possible photodegradation of decaBDE. The mussels were collected approximately 24 hours after the last addition of food (green algae) to allow sufficient time for depuration of any particulate matter present in the gastro-intestinal tract prior to analysis. The levels of decaBDE found in the mussels were displayed graphically in the paper but the levels appeared to be reasonably constant after 48 hours' exposure. There was also evidence for the presence of lower PBDE congeners in the mussels after 168

3.3.1.1 Field studies

1) As described in Section 3.1.3, a series of experiments have been conducted in a freshwater lake as part of a Canadian government-funded project (Orihel et al., 2009 [ABST] and Muir, 2011 [ABST]). One of these experiments (Experiment 2) exposed fish (Yellow Perch *Perca flavescens*) and invertebrates to decaBDE under natural field conditions. Fish captured from the treated mesocosms in October 2008 (three months post-treatment) had higher carcass concentrations of decaBDE on average than fish captured from the control mesocosm, although there was a high degree of variation among individuals. PBDE concentrations in control fish were below the detection limit except for tri- and tetraBDEs, which were present at concentrations below about 1 ng/g dry weight. Fish from the "medium" and "high" mesocosms had higher concentrations of tetra- to nonaBDEs than both the control and "low" mesocosms. For the "high" mesocosm, addition of 2.3 g of decaBDE to a water volume of 180 m^3 resulted in fish concentrations of hepta- and hexaBDEs around 5-10 ng/g dw in total after three months. In contrast, tetraBDEs seemed to be more prevalent in the "medium" mesocosm. This observation suggests that fish were either accumulating lower PBDE congeners formed through degradation of decaBDE in their environment, or taking up decaBDE and debrominating this compound within their bodies (or both). The results relate to carcass concentrations only, and inclusion of liver results (currently unavailable) might mean that the total fish concentrations were higher.

2) An investigation of decaBDE transformation in fish under field conditions has also been performed by La Guardia et al. (2007). (This study was not summarised in previous EU risk assessment reports.) The study analysed the concentrations of triBDEs to decaBDE in samples of (activated) sewage sludge from an industrial wastewater treatment plant in the United States, along with samples of surface sediment and biota from the receiving water system. Samples were collected in November 2002 and November 2005. Biota samples included crayfish (*Cambarus puncticambarus*; five samples in 2002), Chub (*Semolilus atromaculatus*; six samples in 2002), and Sunfish (*Lepomis gibbosus*; thirteen samples in 2002 and twenty-two samples in 2005). The fish and crayfish were kept in holding tanks for 72 hours prior to analysis to allow depuration of gut contents to occur. A single composite sample of each species was extracted and purified using size-exclusion chromatography, then analyzed for PBDEs using GC/MS in ECNI mode and electron ionisation (EI) mode.

   The levels of PBDEs found in the samples are summarised in Table 12 (the study also included several individual tri- to hexaBDE congeners but these are not shown in the table):

   - The PBDE congener pattern found in the sludge samples closely resembled that in a commercial pentaBDE product. The authors therefore concluded that debromination of decaBDE during the wastewater treatment process was unlikely to be a major process contributing to the levels of lower PBDE congeners found in the sludge.

   - The congener profile in the sediment downstream of the wastewater treatment plant was broadly similar to that in the sludge and it was concluded that minimal debromination of decaBDE was occurring in the sediment.

   - Twenty-three PBDEs were detected in the biota samples, with the congeners BDE-47 (tetraBDE), BDE-153 (hexaBDE), BDE-196, BDE-201, BDE-202, BDE-203 (octaBDEs) and BDE-206, BDE-207 and BDE-208 (nonaBDEs) detected in every

hours' exposure. These were not determined quantitatively but were thought to be three heptaBDEs, three octaBDEs and three nonaBDEs.

sample. DecaBDE was detectable in crayfish and one of the sunfish samples but was not detectable in Chub. The congener profile in the chub was of interest as it showed some differences with the profiles found in the other species. For example no BDE-99 (a pentaBDE) or BDE-183 (a heptaBDE) were found in chub even though these were present in both the sediments and other species in the same area. The study authors indicated that a similar congener profile had been reported previously for Common Carp (*Cyprinus carpio*) in a study by Hale et al. (2001). Stapleton et al. (2004b) showed that *C. carpio* exposed to BDE-99 and BDE-183 via the diet could metabolise these substances in the gut by at least 10–12% to form BDE-47 (a tetraBDE) and BDE-154 (a hexaBDE) respectively. As both species belong to the same family (*Cyprinidae*), the study authors speculated that both species might possess a similar metabolic capability, and so species-specific differences in metabolic capacity may explain the differences in the congener patters between the various species.

The study authors concluded that the congener profiles provide some evidence for metabolic debromination of decaBDE (or nonaBDEs). For example, although only the Sunfish from 2002 contained detectable amounts of decaBDE, both the Chub and Sunfish samples contained detectable amounts of two octaBDEs (BDE-201 and BDE-202) and three heptaBDEs (BDE-179, BDE-184 and BDE-188) that were not detectable in either the sludge or sediment samples. This hypothesis is further strengthened by the results of the dietary study exposing Rainbow Trout and Common Carp to decaBDE carried out by Stapleton et al. (2006) (see Section 3.3.1.2): the study authors noted that the congener patterns seen in those two species in dietary exposure studies was similar to that seen in Sunfish and Chub, respectively, in this study.

Table 12: Distribution of PBDEs in sediment and biota downstream of a waste water treatment plant (from La Guardia et al., 2007)

Sample		**Concentrations**							
		DecaBDE	**NonaBDEs**			**OctaBDEs**			**HeptaBDEs**
		BDE-209	**BDE-208**	**BDE-207**	**BDE-206**	**BDE-203**	**BDE-197**	**BDE-196**	**BDE-183**
November 2002									
Sewage sludge (µg/kg dry weight)		58,800	726	1,340	27,400	1,190	993	1,600	310
Sediment (µg/kg organic carbon)	0.2 km upstream	36,800	not detected	not detected	not detected	not detected	not detected	not detected	not detected
	0 km (15 m) downstream	1,630,000	3,530	5,810	67,700	434	not detected	380	not detected
	1.3 km downstream	3,150,000	not detected	6,660	84,000	386	not detected	771	not detected
	5.6 km downstream	642,000	577	2,630	24,300	322	156	620	249
	10.8 km downstream	300,000	not detected	945	10,900	166	73	210	125
Chub (µg/kg lipid)		not detected	103	79	94	117	not detected	45	not detected
Crayfish (µg/kg lipid)		21,600	143	1,920	2,650	132	43	200	not detected
Sunfish (µg/kg lipid)		2,880	201	276	411	74	193	65	83
November 2005									
Sewage sludge (µg/kg dry weight)		37,400	295	276	1,490	220	171	202	89
Sediment (µg/kg organic carbon)	0.2 km upstream	33,300	not detected	not detected	not detected	not detected	not detected	not detected	not detected
	0 km (15 m) downstream	181,000	not detected	not detected	11,200	not detected	not detected	not detected	not detected
	1.3 km downstream	2,310,000	not detected	not detected	31,700	not detected	not detected	not detected	not detected
	5.6 km downstream	2,390,000	1,690	6,520	35,500	553	388	1,120	not detected
	10.8 km downstream	247,000	375	544	3,120	not detected	not detected	not detected	not detected
Sunfish (µg/kg lipid)		not detected	67	73	133	20	86	28	77

3) Viganò et al. (2011) assessed the bioavailability and biotransformation of decaBDE in fish from the River Po, Italy. (This study was not summarised in previous EU risk assessment reports.) Twenty-three cyprinid fish (one Chub, *Leuciscus cephalus*; two Barbel, *Barbus* spp.; and the remainder Common Carp, *Cyprinus carpio*) were collected from September 2004 to July 2005 (mostly between March and July 2005) by electrofishing in the middle section of the river, 10–22 km downstream from the confluence of a contaminated tributary, the River Lambro. Fish were placed in tanks and killed by anaesthetic (ethyl p-aminobenzoate), measured for standard length (to 1 mm) and weight (to 0.1 g), and dissected. Fish livers were freeze-dried and analysed for PBDEs.

   Liver samples (1 g) were Soxhlet extracted following addition of labelled recovery standards. Following extraction, the solvent was evaporated to 5.0 ml under a nitrogen stream. Lipids were removed using an automatic gel permeation chromatography system. The collected fraction was concentrated to 1 ml and purified on a multilayer column by elution with 15 ml of n-hexane/dichloromethane (1:1 v/v). One millilitre of toluene was added to the eluent, after which it was further concentrated to 0.1 mL under a nitrogen flow. Separate Soxhlet extractions were carried out for the gravimetric determination of the lipid content for each sample. Instrumental analysis of decaBDE and tri- to octaBDEs (BDE-17, -28, -47, -49, -99, -100, -153, -154, -155, -183, -179, -188, -202) was performed using gas chromatography-mass spectrometry with external standard calibration. Peaks were identified by matching both retention times and MS/MS spectra, whereas peak quantification was based on selected m/z ion values. No correction for recovery was applied to sample results. Procedural blanks (n=3) were analysed and low levels (below 1 ng/g) of BDE-47 and -209 were detected. For these chemicals, the background mean values were subtracted from the concentrations determined in samples. Blank samples spiked with native BDE-209 (20 ng/g, n=2) were run through all steps of preparation and analysis to check whether BDE-179, -188 and -202 were products of degradation in the laboratory. No extra peaks potentially related to such degradation were found. Based on a signal to noise ratio of 10:1, the following instrumental limits of detection were estimated: tri to pentaBDEs, 0.1 ng/g dry weight (dw); hexa- to octaBDEs 0.2 ng/g dw; decaBDE 0.5 ng/g dw.

   In contrast to sediments and invertebrates collected from the same area, no fish sample contained detectable levels of decaBDE. However, all fish livers contained substantially similar PBDE profiles, and in particular four congeners (BDE-154, -179, -188 and -202). BDE-154 was not detected in the middle river section, and the high relative concentration of BDE-154 compared to BDE-153 was thought to indicate possible transformation within the fish from higher molecular weight congeners. Similarly, BDE-179, -188 and -202 were not detected in sediments or invertebrates at this site, and have not been reported from commercial products. They have, however, been identified as transformation products from decaBDE exposure in Rainbow Trout by Stapleton et al. (2006) (see Section 3.3.1.2).

Discussion

Full experimental details are not yet available, so a reliability marking cannot be assigned to the mesocosm studies of Orihel et al. (2009) [ABST] and Muir (2011) [ABST]. However, the absence of significant inputs from other PBDE sources (i.e. commercial penta- and octaBDE products) means that the findings can be clearly related to decaBDE, so they are highly relevant. Fish were found to accumulate significant quantities of hepta- and hexaBDEs (around 5-10 ng/g (µg/kg) dry weight in total) over the course of several months following exposure to decaBDE (at a nominal loading that was lower than that encountered in polluted sediments in Europe). This might be a minimum concentration since liver results are not yet available. This observation suggests that either fish were accumulating lower PBDE congeners formed through

degradation of decaBDE in their environment, or taking up decaBDE and debrominating this compound within their bodies (or a combination of both).

The studies of La Guardia et al.(2007) and Viganò et al. (2011) provide some indirect evidence that fish species might be capable of transforming decaBDE to at least heptaBDEs. Due to the mixture of PBDEs present in the fish tissues and surrounding media, it is not possible to estimate the contribution that decaBDE makes to the concentrations of lower PBDE congeners that were detected.

3.3.1.2 Laboratory studies

a) Kierkegaard et al. (1997 [ABST] and 1999) investigated the uptake of decaBDE by Rainbow Trout (*Oncorhynchus mykiss*) from food. The test substance was a commercial flame retardant (Dow FR-300-BA; the actual composition of this substance was not given in the paper[51]). The substance was purified on a charcoal column prior to use to remove planar compounds. The fish used in the test were juvenile Rainbow Trout which were kept in a continuous flow of charcoal-filtered brackish water at temperatures corresponding to those found outdoors in Sweden over the months June to September. The food used in the study was cod (*Gadus morhua*) from the Barents Sea. The cod (excluding gonads, gall bladder and liver) was homogenised and mixed with an equal volume of 3% gelatine solution. The test susbstance was dissolved/suspended in corn oil and then mixed with the cod/gelatine solution. The mixture was then air dried and frozen until needed. The doses of decaBDE used in the experiment ranged between 7.5 and 10 mg/kg body weight/day. The fish were sampled for biological and chemical investigation after 16, 49 and 120 days. A further group were exposed for 49 days, followed by a 120 day depuration period. Fish were starved for 24-48 hours prior to sampling.

   During the test, the lipid concentration in the Rainbow Trout muscle decreased from 3.3 to 1.3% in the exposed fish and 3.9 to 0.97% in the control fish and so the uptake of decaBDE in the fish was measured on a fresh weight basis. After 16 days' exposure, the mean muscle concentration of decaBDE was found to be 10 µg/kg fresh weight. The muscle concentration of decaBDE was found to increase with exposure time, reaching a level of 38 µg/kg fresh weight after 120 days. It is possible that steady state was not reached during the exposure part of this study. The concentration found in the livers of exposed fish exceeded those of the muscle (levels in liver were 560 µg/kg fresh weight after 16 days and 870 µg/kg fresh weight after 120 days). In the control fish, 4 out of the 34 muscle samples analysed showed traces of decaBDE (level <8% of the corresponding exposed fish). In the depuration phase of the experiment, the levels of decaBDE were found to decrease by a factor of two on a fresh weight basis after 71 days, but no decrease in the levels was observed when the results were expressed on a lipid basis.

   The concentrations of some hexa-, hepta-, octa- and nonaBDE congeners were found to increase over the exposure period in both muscle and liver in the exposed fish but not the controls[52]. Some of these congeners were not detectable in the commercial decaBDE product used in the study and it was thought that their presence was a result of either a metabolic process or an efficient absorption process of trace amounts initially present in the food/commercial product used. The study was not able to distinguish between these two possibilities. The registrant does not assign a validity mark to this study.

[51] The composition of a product with this name was reported to be 77.4% deca-, 21.8% nona- and 0.8% octaBDE by Norris et al. (1973 and 1974) although the composition is likely to have changed since then.

[52] There was evidence that lower PBDEs were present in both the liver and muscle (e.g. 2,2',4,4'-tetraBDE; 2,2',4,4',5-pentaBDE; 2,2',4,4',6-pentaBDE), but these congeners were also present at similar concentrations in control fish, and so were not related to the decaBDE treatment (i.e. they were not metabolic products).

b) Stapleton et al. (2002 [ABST] and 2004a) exposed Common Carp (*Cyprinus carpio*) to decaBDE via spiked food. The test was carried out using juvenile fish (approximately 100 mm in length) and the fish were randomly assigned to one of five 132-litre polyethylene tanks. Two replicated tanks were used for the control population and three replicate tanks were used for the exposed population. The water used in the test was filtered well water at 22°C and this was provided at a constant flow rate of 1 litre/minute (giving a hydraulic residence time of around 2 hours). Aeration via air stones was provided in the tanks to maintain the dissolved oxygen concentration. The fish were acclimated to the test system for one week (during which they were fed a clean diet) prior to exposure to contaminated food.

The decaBDE used in the experiment was >98% pure (no information was given on the identities of any impurities present) and was dissolved in cod liver oil. The fish diet was a homogenised mixture of blood worms (80% by mass) and fish food pellets (20% by mass). The cod liver oil solution (20 ml) was then mixed into the food to give a decaBDE concentration of 940 µg/kg wet weight. Control food was prepared in a similar way, but pure cod liver oil was added to the homogenised food.

Fish were fed either the spiked (exposed population) or control (control population) diet at a rate of 1 g/day/fish (this corresponded to a daily dose of decaBDE of approximately 40 µg/kg body weight) for 60 days. After the 60-day exposure period, all the fish were fed the control diet for a further 40 days in order to monitor the depuration.

One fish from each tank was sampled on days 0, 5, 10, 20, 30, 45, 60, 69, 85 and 100 of the experiment. The stomach cavity contents of the fish were discarded and the livers (pooled samples for the exposed and control populations) and the remaining whole body (individual samples) were analysed for the presence of PBDEs.

Growth rates were found to be statistically significantly reduced (p=0.05) in the exposed population (growth rate $5.4\times10^{-3}\pm 2.0\times10^{-3}$ day^{-1}) compared with the control population (growth rate $7.7\times10^{-3}\pm1\times10^{-4}$ day^{-1}). In addition the lipid contents of whole fish tissues were also found to be statistically significantly reduced (p=0.05) in the exposed population (lipid content 1.9±0.8%) compared with the control population (2.7±1.0%) based on the average of all fish at all time points throughout the exposure.

No decaBDE was found in the whole fish tissues of either the exposed or control population at any sampling time (the detection limit was 1 µg/kg wet weight). However, around seven peaks were found in the chromatograms from the exposed fish that were not present in the chromatograms from the control fish. Two of these peaks were positively identified as 2,2',4,4',5,6'-hexaBDE (BDE-154) and 2,2',4,4',6,6'-hexaBDE (BDE-155), and the other five peaks were identified as an unknown pentaBDE, an unknown hexaBDE, two unknown heptaBDEs and an unknown octaBDE[53]. The same seven PBDE congeners were also found to be present in the liver samples. The levels of total PBDEs in the liver were consistently higher than found in the whole fish tissue during the exposure period.

The concentrations of these congeners were found to increase throughout the exposure period. The concentrations of some (e.g. the unknown pentaBDE) were found to still increase for up to 10 days after the exposure stopped before decreasing, suggesting that there was an ongoing debromination of body stores of PBDEs. Conversely, the concentration of the unidentified octaBDE was found to decrease immediately once the exposure to decaBDE finished. During the depuration period, the tissue concentration of some of the congeners was found to be variable, but half-lives of around 50 days and 35

[53] The unidentified hepta- and octaBDE congeners were determined to be BDE-188, -202 and -197 by Stapleton et al. (2006).

days were estimated for BDE-155 and BDE-154 respectively. 'Minimal' levels of 2,2',4,4'-tetraBDE or 2,2',4,4',5-pentaBDE were found in both the control fish and the exposed fish.

A mass balance calculation was carried out based on the presence of BDE-154 in the fish. The concentrations of this congener in the fish were up to 35 ng/fish by day 60 of the exposure. This congener was not, however, detected in the spiked food (detection limit was 0.03 ng/g wet weight) and so the maximum amount of BDE-154 that could have come from the food was around 2 ng/fish (assuming the detection limit represents the upper limit of the concentration in food, the substance was 100% absorbed and the daily feeding rate was 1 g food over 60 days). Thus it was concluded that the presence of this substance in the fish could not have been the result of impurities present in the food. The mass balance for the other congeners detected was not provided.

Based on the concentrations of the PBDE congeners in the fish it was estimated that at least 0.44% of the total dose was absorbed (the actual absorption could have been higher than this figure if other metabolites are also formed). This was in reasonable agreement with the absorption seen in the Kierkegaard et al. (1999) study (summarised above). This is equivalent to an absorbed dose of at least 248 ng/fish. The formation of up to 35 ng/fish of BDE-154 (i.e. a hexaBDE) by day 60 therefore represents a conversion of around 14% w/w.

Since the amounts of the lower molecular weight PBDE congeners exceeded those which could have accumulated as a consequence of selective uptake from the food, the authors concluded that their presence in the exposed fish indicated that debromination of the decaBDE was occurring.

This is a reliable study, which provides good circumstantial evidence for metabolic debromination of decaBDE in fish down to hexaBDEs. Although it did not demonstrate metabolism directly, this was investigated in a subsequent experiment (see below). The registrant does not assign a validity mark to this study.

c) In a follow-up study to Stapleton et al. (2004a), Stapleton et al. (2006) investigated the debromination of decaBDE using *in vivo* and *in vitro* experiments. The decaBDE used in the study had a purity of 98.7%.

The *in vivo* studies were carried out using Rainbow Trout (*Oncorhynchus mykiss*) of average weight 91.2 g. Sixty fish were randomly assigned to one of four flow-through tanks. The fish (n = 45) in three of the tanks were fed a spiked diet for five months, and the fourth tank acted as the control. The spiked food was prepared by firstly dissolving decaBDE in cod liver oil. This was then mixed with crushed food pellets (lipid content ~10%) and a 3% gelatine solution was added to produce a solidified food source. The final decaBDE concentration in the food was ~940 µg/kg wet weight. Three sub-samples of the spiked food were analyzed for the presence of lower molecular weight PBDE congeners to determine if any degradation of decaBDE had occurred during the food preparation. The feeding rate used was 1% of the fish body weight/day for five days/week. At various time points during the study, one fish from each tank was sampled and analysed for the presence of PBDEs.

The concentration of decaBDE in the fish food was found to remain constant during the entire experiment (the mean concentration at the start of the test was 939 µg/kg, compared to 936 µg/kg at the end). The liver was found to show the highest level of accumulation of decaBDE in the exposed fish, increasing from not detectable levels at the start to a mean concentration of 401 µg/kg wet weight by the end of the test. The levels in whole body homogenates were found to be much lower than in the liver (reaching around 5.3 µg/kg wet weight after five months' exposure). The authors concluded that these data did not result solely from differences in lipid content (the mean lipid content of the liver was 2.3% compared with 4.5% for the whole body homogenates) and so suggested that the liver acts as a sink for decaBDE.

Serum samples were also analysed. Prior to the start of the experiment, the level of decaBDE in serum was <2.4 µg/kg. Serum samples collected from the exposed population on days 57, 98 and 112 had serum concentrations of decaBDE of 26-40 µg/kg, and these concentrations did not change significantly over the last two months of the experiment. It was concluded that steady state in all tissues and serum appeared to have been reached after about 3.5 month's exposure.

As well as decaBDE, several lower PBDE congeners were detected in the exposed population. The congeners found included all three possible nonaBDEs (BDE-206, -207 and -208), six octaBDEs (two of these were identified as BDE-201 and -202) and a small fraction of heptaBDEs (the major congener present was identified as BDE-188, plus three others). None of these congeners could be detected in the spiked or control food. These congeners were first detectable in the exposed fish on day 10 and the concentrations of the octa- and heptaBDE congeners were found to increase throughout the exposure period. The concentrations of the two main nonaBDE congeners found also increased during the first four months of the experiment, but then appeared to decrease over the last month. The reason for this decrease is unclear. Analysis of whole body homogenates on day 112 of the study indicated that around 69% of the PBDEs present were nonaBDEs (~37% of the total) and octaBDEs (~32% of the total). Hepta- and hexaBDE congeners comprised about 2% and less than 2% of the total PBDE body burden, respectively, after 112 days.

Analysis of intestinal tissues indicated that the same hepta-, octa- and nona- congeners found in the whole body homogenates were also present in the intestinal tissue. This raises a possibility that at least some of the transformation could have occurred in the gut prior to systemic circulation.

To check if the accumulation of these congeners was a result of impurities present in the spiked food mixtures, theoretical calculations were carried out assuming that the maximum amount of 2,2',3,3',5,5',6,6'-octaBDE present in the food was 0.03 ng/kg (the limit of detection) and that the trout accumulated 100% of the dose. Under these assumptions, the maximum body burden of this octaBDE in the fish would have been 2.4 ng per fish. The measured data indicated that by day 10 the amount of this congener present in the fish was 3.3 ng per fish, and by the end of the experiment had reached 506 ng per fish. The authors therefore concluded that it was very unlikely that this congener had accumulated from impurities in food, and so must have been formed as a metabolite of decaBDE in the fish.

The total assimilation of decaBDE by the fish was estimated by summing the body burdens of the major congeners found to be present in the body of the fish (approximately 2,550 pmoles). The total amount of decaBDE fed to the fish over the period of the study was 80.4 nmoles. Thus the fish assimilated at least 3.2% of the dose. This figure rose to 3.7% if the liver was included in the calculations. It should be noted that these figures take into account only the PBDE metabolites; if other metabolites were also formed (e.g. hydroxylated or covalently bound metabolites), the actual assimilation could have been higher.

The *in vitro* experiments were carried out using microsomal preparations from Rainbow Trout and Common Carp (*Cyprinus carpio*) livers. The microsomal fractions were incubated with 15 pmoles of decaBDE/mg protein for 1 and 24 hours at 25°C. The metabolic activity of the microsomes was verified by measuring ethoxyresorufin O-deethylase and the incubation mix was supplemented with 100 µM nicotinamide adenine dinucleotide phosphate (NADPH), although no NADPH regenerating system was provided. The results were as follows:

- Debromination of decaBDE was evident in the Rainbow Trout liver microsomes, and the nona- and octaBDE congeners identified were identical to those found in the *in vivo* experiments. Around 22% conversion of decaBDE to these products was evident after 24 hours' incubation.

- Debromination was found to be faster and more extensive in the carp liver microsomes, with nona-, octa-, hepta- and hexaBDEs (2,2',4,4',5,6'-hexaBDE (BDE-154) and 2,2',4,4',6,6'-hexaBDE (BDE-155)) formed after 24 hours' incubation. Around 65% of the decaBDE was debrominated overall, and 30% debrominated to hexaBDEs. The nonaBDEs did not accumulate. This pattern is consistent with that observed in fish exposed *in vivo* via the diet.

The authors concluded that their results supported the hypothesis that deiodinase enzymes were catalyzing debromination of decaBDE; however, they also cautioned that it was not possible to rule out the concurrent or alternative action of oxidative cytochrome P450 enzymes[54].

Overall, the results of this reliable study provide convincing evidence that decaBDE is metabolised in fish to form hepta- and hexaBDE congeners. This was shown conclusively in the *in vitro* experiments with both trout liver and carp liver extracts, and a very similar pattern of metabolism was also evident in the *in vivo* experiments. The amounts of hepta- and hexaBDEs formed in the *in vivo* experiments with Rainbow Trout represent around 2% of the total PBDEs present after 112 days. The registrant does not assign a validity mark to this study.

d) Nyholm et al. (2008a) performed a dietary exposure study with Zebrafish (*Danio rerio*). (This study was not summarised in previous EU risk assessment reports.) Feed contaminated with various brominated substances (including decaBDE, a heptaBDE (BDE-183), a triBDE (BDE-28) and eight other non-PBDE brominated flame retardants; purity was not stated) was prepared by adding a mixture of the substances in ethanol to freeze-dried chironomid feed. The ethanol was then allowed to evaporate. Two nominal concentrations were prepared, a high dose where the concentration of each substance was 100 nmol/g dry food (equivalent to a decaBDE concentration of 96 mg/kg dry food) and a low dose where the concentration of each substance was 10 nmol/g dry food (equivalent to a decaBDE concentration of 9.6 mg/kg dry food). Groups of 23 males and 23 females were used for each exposure concentration and were fed at a rate of 2% of their body weight per day (to ensure the feed was completely consumed, half the feed was given in the morning and half in the afternoon) for up to 42 days. Fish were sampled on days 0, 3, 7, 14, 28, 35 and 42 of the experiment and eggs were collected on days 0, 2-3, 6-7, 13-14, 27-28, 34-36 and 41-42. The average lipid contents of the fish and eggs during the study were 3.36% and 0.47% respectively. The lipid content of the feed was not given.

Three hexaBDEs accumulated in eggs and fish (the actual congeners were not identified). The amount of one of these congeners in the spiked food could have accounted for the amount accumulated in the fish. For the other two, however, the cumulative exposure via impurities in the spiked food was less than 1% of the level measured in the fish after 42

[54] Benedict et al. (2007) investigated the mechanism of 2,2',4,4',5-pentaBDE (BDE-99) debromination to 2,2',4,4'-tetraBDE (BDE-47) in Common Carp (*Cyprinus carpio*) using liver and intestinal components. It was found that intestinal microflora are not responsible for BDE-99 debromination. Rather, it is an endogenous process which occurred with approximately equal activity in intestine and liver microsomes. Debromination was inhibited by reverse thyronine (rT3). The presence of NADPH in the microsomal assay did not significantly ($p>0.05$) affect BDE-99 debromination, which suggested that cytochrome P450 enzymes were not the main debrominating pathway for BDE-99. Noyes et al. (2010) characterized the biotransformation of BDE-99 using *in vitro* hepatic sub-cellular fractions prepared from individual adult *C. carpio*. Debromination rates to form BDE-47 were generally higher in the microsomal fraction than in the cytosolic fraction. Iodoacetate and the two thyroid hormones, reverse triodothyronine (rT3) and thyroxine (T4), significantly inhibited the debromination of BDE-99 in microsomal fractions. These findings support the hypothesis that thyroid hormone deiodinase enzymes may be catalyzing the metabolism of PBDEs in fish liver tissues.

Browne et al. (2009) conducted similar experiments with Chinook Salmon (*Onchorhynchus tshawytscha*) liver microsomal fractions, and found no debromination of BDE-99 to BDE-47, but rather a slow transformation to BDE-49 (2,2',4,5'-tetraBDE). This reaction was not NADPH-dependent, indicating a lack of cytochrome P450 involvement. By contrast, omission of the reductant dithiothreitol (DTT) from microsomal preparations resulted in a lack of BDE-99 debromination, suggesting the involvement of a hepatic reductase(s) or deiodinase.

days' exposure. This suggests that they were metabolically derived in the fish from debromination of a heptaBDE (BDE-183) and/or decaBDE.

e) Nyholm et al. (2008b) performed a second study on male Zebrafish (*Danio rerio*) using a similar dietary exposure method with the same substance mixture as used by Nyholm et al. (2008a). (This study was not summarised in previous EU risk assessment reports.) Groups of fish were exposed to nominal concentrations of either 1 or 100 nmol/g food for each component (equivalent to a decaBDE concentration of either 0.96 mg/kg dry weight or 96 mg/kg dry weight in the food) for a total of 42 days. This was followed by a 14-day elimination period where the fish were fed an uncontaminated diet. Fish were analysed for the presence of decaBDE and possible metabolites by GC/MS on days 0, 3, 7, 14, 28, 35 and 42 of the uptake period and days 7 and 14 of the elimination period (the fish were sampled 24 hours after feeding, and a composite sample of two whole fish was analysed on each occasion).

   Fish concentrations are shown graphically in the paper. Uptake efficiency for decaBDE was low (< 1%), and the elimination half-life was estimated at 6.5 days. In the high dose group the concentration of decaBDE reached around 0.08 nmol/g wet weight in the fish after 42 days' exposure and the plot indicated that steady state was being approached after this time. The measured concentration of decaBDE in the food for this group was 72 nmol/g dry weight. HexaBDEs were also detectable in the exposed fish but as before, it is not possible to ascribe the presence of these lower congeners solely to exposure to decaBDE.[55]

f) Lebeuf et al. (2004 [ABST] and 2006) investigated the possible metabolism of decaBDE in Atlantic Tomcod (*Microgadus tomcod*) following pre-treatment with a cytochrome P4501A inducer with a high potency to induce the liver detoxification system (PCB-126). The fish used in the study (175-250 mm) were captured in the St. Lawrence estuary in November 2001 and acclimated to laboratory conditions (salinity 30 ppm and temperature of 6-7°C) until the start of the experiment the following May. During this period the fish were fed *ad libitum* with frozen Capelin or Rainbow smelts twice per week. At the start of the test eight groups of 25 fish were placed into 500 litre fibreglass tanks. Fish from half the tanks were then anaesthetized and injected with PCB-126 (dose 25 ng/g of fish in corn oil; fish from the remaining tanks received a dose of corn oil alone). After three weeks, the fish from two out of the four tanks that had received PCB-126 and the fish from two out of the four tanks that had received corn oil alone were injected with decaBDE (dose 400 ng/g fish; fish from the remaining tanks received a dose of corn oil alone). After a further seven weeks, groups of five to six male fish from each tank were sampled and analysed for the presence of decaBDE and several lower PBDE congeners (di- to nonaBDEs).

   Analysis of the decaBDE used in the study found that the substance had a purity of around 96%. The main impurities identified included 2,2',4,4'5,5'-hexaBDE (0.00024-0.00041% w/w), 2,2',3,4,4',5,6'-heptaBDE (0.0050-0.0055% w/w) and 2,2',3,4,4',5,5',6-octaBDE (0.032-0.034% w/w), and all three possible nonaBDE congeners, along with four unidentified hepta- and three unidentified octaBDEs.

   At the end of the exposure period, the livers of control fish were found to contain a total of twelve identifiable PBDEs. The total concentration of these congeners in the control fish was 224 µg/kg wet weight, with three congeners (2,2',4,4-tetraBDE, 2,2',4,4'5-pentaBDE and 2,2',4,4',6-pentaBDE) comprising around 80% of this total. In addition, two methoxy-derivatives of PBDE (2'-methoxy-2,3',4,5'-tetraBDE and 6-methoxy-2,2',4,4'-tetraBDE) were also present in the control fish livers.

   The level of decaBDE present in liver of the exposed fish at the end of the study was 421 µg/kg wet weight in the fish pre-exposed to corn oil alone and 420 µg/kg wet weight in

[55] A further dietary study exposing zebra fish (*Danio rerio*) to decaBDE was briefly reported by Rattfelt et al. (2006) [ABST], but no data on transformation were reported.

the fish pre-exposed to PCB-126. The transfer efficiency of decaBDE to the liver was estimated to be 5.4±5.6%. In addition to the substances found in the control fish, the livers of the exposed fish were also found to contain measurable quantities (either systematically or sporadically) of the following congeners: 2,3',4,4',5-pentaBDE (sporadically), 2,2',3,4,4',5'-hexaBDE (sporadically), 2,3,3',4,4',5,6-heptaBDE (sporadically), 2,2',3,4,4',5,5',6-octaBDE (systematically), three unidentified octaBDE congeners (systematically) and all three possible nonaBDEs (systematically). Treatment with decaBDE (both with and without pre-treatment with PCB-126) did not lead to any significant enrichment of the substances found to be also present in the control fish. With the exception of the sporadic occurrences and one unidentified octaBDE, the pattern of congeners found in livers of the exposed fish could be explained by the uptake of impurities from the decaBDE used. It was therefore concluded that the presence of these substances most likely resulted from impurities in the decaBDE, but that it could not be excluded that some congeners resulted, at least in part, from biotransformation and/or from thermal degradation of nona-/decaBDE during the analytical procedure. There was also some evidence that, in the fish pre-exposed to PCB-126, enhanced metabolism of 2,2',3,4,4',5,5',6-octaBDE may have occurred. Overall it was concluded that this fish species exhibited very limited capacity to metabolise decaBDE to lower PBDE congeners.

g) Tomy et al. (2004) investigated the uptake of decaBDE by juvenile Lake Trout (*Salvelinus namaycush*) from food. Groups of fish (initial mean weight 55 g; 70 fish per treatment group) were exposed to a diet containing thirteen PBDE congeners (the congeners ranged from tri- to decaBDE). A known amount of each congener was mixed with corn oil and then added to a blender containing commercial fish food. The mixture was then stirred gently for 20 minutes after which an aqueous gelatin binder was added. The mixture was then stirred again until a firm consistency was obtained (after around a further 20 minutes). The spiked food was then air dried for 40 minutes, extruded through a 4 mm diameter "noodler", dried at 10°C for 48 hours and then crushed into pellets. The control food was prepared in the same way without the addition of the PBDEs. Two dosing levels were used (~2.5 µg/kg food per congener and ~25 µg/kg food per congener) along with a control diet containing no added PBDEs. The individual congeners used in the study each had a purity of >96%. The concentration of decaBDE in the food was found to be 3.4 µg/kg dry weight in the low dose food and 27.5 µg/kg dry weight in the high dose food.

The exposure part of the study was carried out for 56 days and this was followed by a 112-day depuration period (during which the fish were fed uncontaminated food). The feeding rate in the study was 1.5% of the mean weight of the trout (adjusted after each sampling period). At various times during the study the concentrations of decaBDE present in fish muscle were determined. These concentrations were corrected for the concentrations found in the control fish, lipid normalised and corrected for growth dilution.

Possible evidence for transformation included:

- The concentration of some PBDE congeners (e.g. 2,2',4,4',6-pentaBDE in the high dose experiment only) increased during the depuration phase, suggesting that they were being formed inside the fish.

- The chromatographic elution patterns indicated that a number of PBDE congeners were present in the exposed fish that were absent from the fish food (the detection limit for these congeners in the food was not given and it is possible that they were present at concentrations below the detection limit). It was noted in the paper that one of the congeners that was absent from the food (2,2',3,4,4',6'-hexaBDE) could only be derived from decaBDE out of the congeners added to the food.

- Some of the depuration half-lives for certain lower PBDE congeners were longer than expected. One possible explanation suggested by the authors was that they were being formed in the fish from other PBDEs.

It should be noted that the lack of liver measurements might have underestimated transformation to lower molecular weight PBDEs. The paper also performed an analysis to determine whether impurities present in the decaBDE test substance could have explained the uptake pattern seen. The maximum concentration of decaBDE determined in the fish after 56 days' exposure was ~200 µg/kg. Using this concentration as a basis, a standard solution of the decaBDE used (corresponding to a concentration of 200 µg/kg fish) was analysed for the presence of penta- and hexaBDE congeners. No penta- or hexaBDEs could be detected in this solution and so the authors argued that impurities in the decaBDE test substance could not account for the uptake patterns seen. However, this argument appears to be flawed as it assumes that the accumulation of the penta- and hexaBDE congeners would occur at the same level as for decaBDE. This assumption is incorrect based on the known behaviour of these congeners.

The presence of decaBDE (and several of the lower PBDE congeners) in the control food, and the fact that exposure was to a mixture of PBDE congeners, mean that the results of this experiment are difficult to interpret. Although the authors thought that the results were suggestive of debromination to lower PBDE congeners, the study is considered to be inconclusive. The registrant assigns a validity mark of 'not reliable' to this study.

h) Feng et al. (2010) exposed Rainbow Trout (*Oncorhynchus mykiss*) to decaBDE via a single intraperitoneal injection. (This study was not summarised in previous EU risk assessment reports.) Juvenile fish (about four months old, length ~200 mm and weight ~100 g) were randomly stocked in 250 litre glass tanks, and maintained in aerated de-chlorinated tap water at a constant temperature of 15 ± 2°C, with a photoperiod of 16 hours light:8 hours dark. Fish were acclimated for a week prior to exposure. The test substance was a commercial decaBDE product (purity > 98%) obtained from the Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China. It was dissolved in corn oil to make a 250 mg/l decaBDE solution. Fish in the treatment groups were injected intraperitoneally with the decaBDE solution (50 µl in one group and 200 µl in the second, to give initial decaBDE concentrations of 100 or 500 ng/g fresh weight, respectively). A control group was injected with corn oil only. No information is provided in the paper about the number of fish used for each treatment. The fish were fed once a week (*sic*) with Tetra Pond Sticks (ensuring that the food was consumed within 20–40 seconds), and the experiment was terminated after 28 days. (N.B. This level of feeding is not ideal – for example the OECD Test Guideline 305 for fish bioaccumulation studies recommends feeding once a day. It is not known whether the feeding rate is a reporting error) No information is given about whether there was any water flow in the tanks.

Three replicate tissue samples were prepared from fish randomly sampled from each tank on day 1 and day 28 post injection. To avoid contamination from the gastrointestinal tract, food was withheld from the fish for 24–48 hours before sampling. Blood samples were taken directly from the caudal aorta and collected in centrifuge tubes containing sodium heparin as the anticoagulant. The blood samples were immediately separated into plasma and red blood cells by centrifugation (10,000 r.p.m.) for 15 minutes at 4°C. The viscera of each fish were dissected, and the muscle and liver separated. The muscle, liver and blood samples were stored at –80°C prior to further analysis for 27 PBDE congeners (from mono- to decaBDE), four methoxy-tetraBDEs and four methoxy-pentaBDEs via gas chromatography–mass spectrometry (GC/MS). Two different ionization techniques were used: electron ionization (EI) and electron-capture negative ionization (ECNI). All analytes were identified on the basis of their retention time, relative to authentic standards. To prevent photo-degradation, the samples remained wrapped with aluminum foil. Additionally, one procedural blank was run with every batch of 6–10 samples to assess potential sample contamination (this showed that the sample analysis was free from contamination). The recovery of the surrogate standards in spiked blanks and fish samples ranged from 78.9 to 120.5%, and from 72.6 to 116.8%, respectively.

The detection limit for decaBDE was approximately 1.25 ng/g ww, and for other analytes varied from congener to congener based on the amount of sample and the instrument

sensitivity (0.05–1.25 ng/g in muscle and liver, and 0.25–2.55 ng/g in blood, respectively). Concentrations below the detection limits were assumed to be zero for the subsequent data analysis.

No PBDEs were detected in the control group, whereas PBDE metabolites were observed in both treatment groups. DecaBDE was the dominant congener in both treatments at day 1, but its contribution to the overall PBDE load had reduced substantially by day 28. The highest concentration detected was in muscle tissues in the high dose group, with a mean of 796 ng/g ww on day 1 (standard deviation (s.d.) 38.4 ng/g ww), falling to a mean of 687 ng/g ww (s.d. 30.98 ng/g ww) on day 28. DecaBDE concentrations were much lower in the liver samples, and it was not detected in the blood for either treatment.

Of the 26 other individual PBDEs analyzed, 18 PBDE congeners were detected in the day 1 samples, including one monoBDE (BDE-3), two diBDEs (BDE-7 & -15), five tetraBDEs (BDE-47, -49, -66, -71 & -77), five pentaBDEs (BDE-85, -99, -100, -119 & -126), one heptaBDE (BDE-184), one octaBDE (BDE-197) and two nonaBDEs (BDE-206 & -207) [56]. No tri- or hexaBDEs were observed. In the day 28 samples, one triBDE (BDE-28) was detected, as well as a different heptaBDE (BDE-183). There was no significant difference in PBDE congener patterns between the low and high dose groups in general terms. The most commonly detected lower molecular weight congener was BDE-47, found in 83% of the samples, followed by BDE-49 and -71, which were found in 75% of the samples. BDE-183 and -184 had the lowest detection frequency (below 10%). The highest concentration of the lower molecular weight congeners was for BDE-207 (8.7 – 359 ng/g), followed by BDE-197 (53 – 245 ng/g) and BDE-206 (11 – 128 ng/g). BDE-100 was present in the smallest amounts (0.2 – 1.8 ng/g). The highest concentration of these metabolites occurred in the liver, followed by blood then muscle. The higher molecular weight PBDEs resided mainly in muscle and liver tissues, whereas the other PBDEs resided mainly in blood tissue.

None of the eight analysed methoxylated PBDE metabolites were detected in the control group, but five were detected in the treated fish on day 1, with two more on day 28. The highest concentration was found in blood (and the lowest in muscle). The individual and total congener levels showed an increasing trend over the 28-day exposure period. The predominant congener was a mono-methoxylated tetraBDE, which was detectable in all samples, and also at the highest concentrations (3.31 to 183.8 ng/g). The next most frequently detected congener was another mono-methoxylated tetraBDE (detection frequency 66.7%), although this was present the lowest concentration (2.39 to 115.1 ng/g).

The exposure route used in this study is not environmentally realistic, and the study used internal doses around two orders of magnitude higher than those typically found in environmental fish samples. The reported feeding rate is also very low – it would seem that the fish were starved and so their metabolism might not have been normal. It is therefore unclear whether the observed pattern of degradation is relevant. The study shows that Rainbow Trout can metabolise decaBDE to lower molecular weight PBDE congeners (including tetra-, penta- and heptaBDEs) as well as methoxylated PBDEs over a 28-day period. The metabolite concentration showed an increasing trend from day 1 to day 28, while deca- and nonaBDE concentrations declined. The metabolite burden in fish exposed via their diet and water is likely to be much lower than suggested by this study over the same time period.

i) Noyes & Stapleton (2010) [ABST] and Noyes et al. (2011) investigated decaBDE accumulation in Fathead Minnows (*Pimephales promelas*) following dietary exposure. (This study was not summarised in previous EU risk assessment reports.) Adult fish received a 28-day dietary treatment of decaBDE at 8.0 ± 0.15 µg/g of food at 5% of their body (wet) weight per day (the food was a commercial feed called Omnivore Gel Diet™). Control fish

[56] The seven congeners not detected were BDE-17, -138, -153, -154, 156, -191 and -196.

received untreated food at a 5% ww/day regimen. No information was provided about the number of tanks or number of fish per tank. Three fish (one male, two females) from each tank were euthanized with on days 0, 14 and 28. In a follow-up study, three separate pools of juvenile fish (28 days old) received a 28-day dietary treatment of decaBDE at 9.8 ± 0.16 µg/g of food at 5% of their body (wet) weight per day, followed by a 14-day depuration period in which they were fed clean food (the feed was frozen *Artemia* spp.). Three separate pools of control fish were fed untreated food at a 5% ww/day regimen. Fish were euthanized on days 0, 14, and 28, and preserved at -80 °C until further processing.

Whole tissue homogenates were extracted using dichloromethane, and biogenic materials were removed using membrane filtration, Gel Permeation Chromatography (GPC)/High Performance Liquid Chromatography (HPLC), and Florisil Chromatography. PBDEs were quantified in the whole body homogenates of each fish (n = 3 per sample day) using gas chromatography/mass spectrometry operated in electron capture negative ionization mode (GC/ECNI-MS).

Adult fish were found to have accumulated decaBDE after 28 days at a mean whole body concentration of 4.7 ± 1.6 ng/g ww. The treated fish were also found to contain other PBDE congeners, dominated by hexa- to octaBDEs. The dominant PBDEs detected were: BDE-154 (a hexaBDE) at 12.2 ± 3.0 ng/g ww; an unknown hexaBDE congener at 10.1 ± 2.6 ng/g ww); BDE-179 (a heptaBDE) at 13.1 ± 3.6 ng/g ww; BDE-188 (a heptaBDE) at 16.6 ± 2.6 ng/g ww; BDE-201 (an octaBDE) at 1.1 ± 0.3 ng/g ww; and BDE-202 (an octaBDE) at 8.6 ± 2.3 ng/g ww. All of the mean PBDE concentrations in the treated fish (n = 6) were statistically significantly different from the control fish ($p<0.05$).

Juvenile fish also accumulated decaBDE in tissues, and penta- to octaBDEs were found to be present at higher concentrations than in controls. BDE-154 was again the dominant lower PBDE congener.

This study provides some evidence of debromination in another fish species, although the lack of information on the test substance means that it is not possible to rule out the possibility that the lower molecular weight PBDE accumulation was due to trace impurities.

j) Roberts et al. (2011) investigated the *in vitro* hepatic metabolism of eleven individual PBDE congeners (tri- to decaBDE) in three different fish species: Rainbow Trout (*Oncorhynchus mykiss*), Common Carp (*Cyprinus carpio*), and Chinook Salmon (*O. tschwatcha*). (This study was not summarised in previous EU risk assessment reports.) The influence of PBDE structural characteristics (i.e. bromine substitution patterns) on metabolism was also evaluated. Six of the eleven congeners (BDE-99, -153, -183, -203, -208 and -209) were metabolically debrominated to lower PBDE congeners; each contained at least one meta-substituted bromine. Metabolites were not detected for congeners without one meta-substituted bromine (e.g. BDE-28, -47 and -100). Metabolite formation rates were generally 10 to 100 times faster in *C. carpio* than in the other two species. BDE-49, -101, -154 and -183 (a tetra-, penta-, hexa- and heptaBDE, respectively) were the major metabolites observed in all three species, with BDE-47 (another tetraBDE) additionally detected in *C. carpio*. Carp liver demonstrated a preference for meta-debromination, while the other two species debrominated *meta*- and *para*-bromine atoms to an equal extent. Glutathione-S-transferase and deiodinase activity were compared among all three species. Carp exhibited a preference for meta-deiodination of the thyroid hormone thyroxine, which was consistent with the preference for meta-debromination of PBDEs observed in carp.

Overall, this study provides good evidence for the potential of three fish species to debrominate higher molecular weight PBDEs (including decaBDE) to lower molecular weight PBDEs, including penta- and tetraBDEs. However, this does not provide any indication of the actual degree of debromination in whole fish following exposure to decaBDE.

k) Zeng et al. (2011) studied the gastrointestinal absorption, metabolic debromination and hydroxylation of three commercial PBDE products in juvenile Common Carp (*C. carpio*). The fish were exposed via their diet to each product separately, over 20 days, at a feeding rate

of 100 – 150 µg/day/fish, depending on the product. The absorption rate of the pentaBDE product was higher than that of the octa- and decaBDE products. However, there were no significantly positive relationships between the number of bromine atoms and the absorption rate, especially for congeners with more than six bromine atoms. The major congeners in fish carcass were: BDE-47 and -100 in the pentaBDE exposure; BDE-154, -155, -149 and -153 in the octaBDE exposure; and BDE-154, -155, -149, -188, -179 and -202 in the decaBDE exposure.

A number of congeners that were not detected in the administered food were found in the faeces for both octa- and decaBDE exposed fish (including penta- and hexaBDEs), indicating that debromination had occurred. Twenty congeners (mostly tri-, tetra- and pentaBDE congeners) were found in fish carcass dosed with the commercial octaBDE product which were not detected in the administrated food. These fish mainly accumulated four hexaBDE congeners. The congeners presented in the fish carcass exposed to the commercial decaBDE product were similar to those detected in the fish carcass exposed to the octaBDE product, including hexaBDEs.

Eleven identified and several unidentified hydroxylated PBDE congeners were found in the pentaBDE-exposed fish. No hydroxylated PBDE congeners were found in the serum samples from decaBDE-exposed fish. No methoxylated PBDE congeners were detected in any serum samples.

l) Kuo et al. (2010b) investigated decaBDE uptake in juvenile Lake Whitefish (*Coregonus clupeaformis*). (This study was not summarised in previous EU risk assessment reports.) Fish were fed a diet containing decaBDE at four nominal concentrations (control, 0.1, 1, and 2 µg/g diet) for 30 days. Livers and carcasses were analyzed for eleven PBDE congeners (BDE-47, -99, -100, -153, -154, -196, -197, -206, -207, -208, and -209) and daily otolith increment width was measured as an estimate of growth before and after exposure. Four congeners (BDE-206, -207, -208, and -209) were detected in livers and carcasses. Hepatic decaBDE concentrations in the 1 and 2 µg/g treatments were significantly higher than in the control group (1.25 and 5.80 nmol/g lipid compared to 0.183 nmol/g lipid). The detection of decaBDE in the tissues of the control group was due to its presence in the base diet. Concentrations of all congeners from the 1 and 2 µg/g groups were higher in livers than carcasses, indicating that the liver was the primary organ of decaBDE accumulation. Compared to the fraction in diets, the molar fraction of decaBDE was lower in livers and carcasses, whereas the fractions of BDE-206, -207, and -208 were higher. These different distributions of PBDE congeners were thought to result from differential absorption and/or metabolism. The authors suggested that BDE-206 could be a major metabolite from decaBDE debromination.

Discussion

A number of laboratory studies have investigated decaBDE metabolite profiles in several fish species. The exposure routes and durations differ, which makes direct comparison difficult, and there are some unexplained differences in metabolic products (for example Feng et al. (2010) did not detect hexaBDEs in *O. mykiss*, unlike Stapleton et al. (2006)). However, taken together, these studies provide convincing evidence that following dietary exposure, some fish species can transform decaBDE to at least hexa- and heptaBDEs, as well as methoxylated and hydroyxlated PBDEs. The yield of these PBDE metabolites is generally low (typically below 5% of the absorbed decaBDE dose in the various studies), but the yield of precursors (i.e. nona- and octaBDEs) is significantly higher. The overall absorption of decaBDE from food is relatively low (up to about 5% in the species studied, although this could be an underestimate if non-PBDE metabolites are formed in significant amounts), so the actual amounts are small. Species differences in the extent of transformation are apparent, and further studies implicate the role of enzymes involved in thyroid hormone homeostasis.

3.3.1.3 Modelling studies

Gandhia et al. (2011) used a "rigorously calibrated", multi-chemical, dynamic fish model to predict the debromination of decaBDE to BDE-154, -100, -99 and -47 (a hexa-, two penta- and one tetraBDE) over a 15-year life period of piscivorous- and non-piscivorous Lake Trout (*Salvelinus namaycush*). (This study was not summarised in previous EU risk assessment reports.) A sensitivity analysis was performed by changing dietary dose, gut absorption efficiency and half-life for generally conservative scenarios. The model predicted that bioaccumulation of these four lower PBDE congeners due to dietary exposure to decaBDE over the 15-year period would be up to ~1000 µg/kg wet weight in non-piscivorous fish under worst-case scenarios. It should be recognised that this modelling exercise only considered four out of the very many possible congeners that are of concern. In addition, other fish species (e.g. cyprinids) appear to be able to metabolise decaBDE more effectively than trout, and so it is possible that this approach could underestimate the potential levels that may be achieved in some fish species.

3.3.1.4 Summary of transformation in aquatic species

Fish can take up decaBDE from their diet, with absorption efficiencies in the region of 5%, and transform it into at least hexa- and heptaBDEs, as well as methoxylated and hydroyxlated PBDEs. The yield of the hepta- and hexaBDE metabolites is generally low (typically below 5% of the absorbed decaBDE dose in the various studies) and the actual amounts are small, but the formation of precursors is more extensive and these could provide an ongoing source over longer time periods. Species differences in the extent of transformation are apparent, and further studies implicate the role of enzymes involved in thyroid hormone homeostasis.

Steady state may take several months to achieve under constant exposure situations. The most persuasive evidence of the relevance of this process in the environment is from mesocosm studies that show that a single decaBDE application of 2.3 g to an aquatic system volume of 180 m^3 gives rise to at least 5-10 µg/kg dw of tetra- to heptaBDEs in fish after three months. Modelling suggests that further accumulation might occur over the life time of the fish.

3.3.2 Transformation in terrestrial species

A number of studies have investigated the uptake and metabolism of decaBDE in birds and mammals. The most relevant studies are summarised in detail below.

3.3.2.1 Birds

Several studies have investigated PBDE congener patterns in tissues of wild birds:

- Chen et al. (2008) measured decaBDE in Peregrine Falcon (*Falco peregrinus*) eggs in the north-eastern United States. (This study was not summarised in previous EU risk assessment reports.) Eggs (n = 114) were collected between 1996 and 2006 (excluding 1997 and 1998), and decaBDE concentrations ranged from 1.4 to 420 ng/g ww. Together with decaBDE, eight nona- and octaBDE congeners comprised 16–57% of total PBDEs in urban eggs and 4.9–53% in rural eggs. BDE-202 (an octaBDE) was detected in over 90% of the eggs, but is not a known component of commercial PBDE products. The concentration of BDE-201 (another octaBDE) was not in proportion with this congener's presence in the commercial octaBDE product. The authors suggested that these two congeners might have been formed *in vivo* from higher molecular weight PBDEs.

- Holden et al. (2008 [ABST] & 2009) studied the PBDE congener pattern in Peregrine Falcon (*Falco peregrinus*) eggs from California. (This study was not summarised in previous EU risk assessment reports.) The same study also appears to have been reported by Park et al. (2009). The study used 95 eggs collected between 1986 and 2007 and the eggs were analysed for a range of PBDE congeners. The paper indicates that most of the egg samples were analysed without the shell but no distinction is made between the samples with and without shell in the rest of the paper. The analytical method involved high resolution gas chromatography coupled with high resolution mass spectrometry and the quality assurance/quality control measures used included minimising exposure to UV light and routine analysis of blank and quality control samples. The recovery for decaBDE was reported to be 59.6% and it is not clear if the concentrations of decaBDE found were corrected for this recovery.

  The median level of decaBDE in the eggs was 0.49 mg/kg lipid. NonaBDEs (sum of BDE-206, BDE-207 and BDE-208) were also present at a median level of 0.18 mg/kg lipid. Also present were octaBDEs (median concentration 0.80 mg/kg lipid), heptaBDEs (median concentration 0.95 mg/kg lipid), hexaBDEs (median concentration 1.95 mg/kg lipid), pentaBDEs (median concentration 1.90 mg/kg lipid) and tetraBDEs (median concentration 0.54 mg/kg lipid). The study authors found that the total PBDE congener profile in the eggs differed markedly from that found in aquatic biota, where the lower PBDE congeners (particularly tetraBDEs and pentaBDEs) tend to dominate.

  The profiles of the major heptaBDEs (BDE-179 and BDE-183), octaBDEs (BDE-196, BDE-197, BDE-201, BDE-202 and BDE-203) and nonaBDEs were compared with the profiles reported for commercial penta-, octa- and decaBDE products. It was found that two congeners (BDE-202 and an unknown heptaBDE[57]) were present in the eggs that were not reported to occur in commercial products. The study authors suggested that this was evidence for biological debromination of decaBDE.

- Park et al. (2008) [ABST] analysed blood plasma collected from nine wild American Kestrels *Falco sparverius* in California for eighteen PBDEs and seven hydroxylated PBDEs using high resolution gas chromatography - high resolution mass spectrometry (HRGC-HRMS) and GC-NCI/MS or EI/MS/MS. DecaBDE comprised 26% (median) of the total PBDE concentration (0.05 to 3.68 ng/g wet weight), followed by BDE-153, -99, -47, -183, -154, and -100. BDE-207, a nonaBDE, was found in almost all samples, and correlated (r^2=0.61) with levels of decaBDE. The ratio of BDE-207 (an nonaBDE) to decaBDE (9% on average) was higher than that of typical commercial products (~0.25%), suggesting that this congener might result from debromination in the birds. Hydroxylated PBDEs were detected at trace levels, with a maximum total concentration of 0.34 ng/g ww (median 0.05 ng/g ww).

There are many uncertainties in interpreting these data given the wide range of sources that the birds could have been exposed to (including different commercial products), and the findings therefore provide only equivocal evidence for transformation. The finding of BDE-202 in the samples might be important, because this congener has been suggested to be a potential marker for debromination of decaBDE (e.g. Gerecke et al., 2005).

Van den Steen et al. (2006 [ABST] and 2007) studied the tissue distribution and metabolism of decaBDE in European Starlings (*Sturnus vulgaris*). Seven adult male birds were exposed via implanted silastic tubes (the silastic implants used were thought to provide a slow release of the chemical over a prolonged period (of around 15 weeks)). The birds were housed in a large outdoor aviary and food and water were provided *ad libitum* (no information on the source of food used was given). The implants were prepared by firstly dissolving the substance in isooctane mixed with peanut oil and then removing the isooctane by gentle heating (40°C)

[57] The Holden et al. (2009) paper is not entirely clear on this point as in one place it is mentioned that this congener has been present in trace amounts in a commercial octaBDE product.

until a constant weight was obtained. The resulting oil was then added to the implants and the implants were inserted under the skin by a small incision to lie alongside the ribs. The exposed group (four birds) each received an implant dose of 46.8 ± 2.2 µg of decaBDE and the control group (three birds) received an implant filled with peanut oil alone. The purity of the test substance (sourced from Wellington Laboratories, Guelph, Canada) was not given but it was indicated that no detectable levels of other PBDEs were present in the spiked solutions used in the implants. Blood samples were taken every three to seven days and after 76 days the amounts of decaBDE (and metabolites) present in pectoral muscle and liver were determined. As the levels present in the samples were generally very low, the tissue samples were pooled in both the exposed group (pooled from two individuals) and control group (pooled from all three individuals) to facilitate analysis. Procedural blank samples (water instead of blood) were included with each batch of samples analysed, and levels found in the samples were corrected for the blank values. In addition to the tissue samples, the amount of decaBDE remaining in the implant was also determined after 76 days. The procedural blank samples gave a low, but consistent response from decaBDE and the concentrations reported in the samples were corrected for this background response. The limits of quantification were set at three times the procedural blank level.

At the end of the exposure period the mean amount of decaBDE remaining in the silastic implants was determined to be 24.2 µg per implant. This shows that about half of the total dose present was released over the 76-day exposure period. During the first half of the exposure period, no significant differences in body mass between the control population and the exposed population was evident. However, during the second half of the exposure period, the body mass of the exposed population tended to be significantly lower than the control population.

The levels of decaBDE in blood of the birds prior to implantation were below the limit of quantification (<0.8 µg/l blood). The levels of decaBDE in control birds during the course of the study were between <0.8 µg/l and 1.0 µg/l. In the exposed birds, the level of decaBDE in blood reached a mean peak level of 16.1 µg/l on day 10. After this time there was a steady decline in the concentration in blood to a mean concentration of 3.3 µg/l by day 76. No lower PBDE congeners were detected in any sample of blood during the exposure period. The half-life of decaBDE in blood was estimated to be around 13 days.

For the muscle and liver samples taken at day 76, the concentration of decaBDE in the control group was below the limit of quantification (<5.6 µg/kg lipid in muscle and <2.9 µg/kg lipid in liver). The levels of decaBDE found in the exposed groups (two pooled samples each from two individuals were analysed) were 430 and 461 µg/kg lipid in muscle and 237 and 269 µg/kg lipid in liver. In addition to decaBDE, other PBDE congeners were found to be present in muscle and liver of exposed birds (similar congener profiles were obtained for both muscle and liver). Many of these congeners were also present in the control birds but it was found that there were marked differences between some of the octa- and nonaBDE congeners present in the exposed birds compared with the control birds.

Discussion

The available evidence is very limited, but suggests that birds might be able to debrominate decaBDE to at least octaBDEs. This is based on chemical analysis of tissue samples collected from the field, and a study of a small number of captive birds that were exposed using a silastic implant over 76 days (2.5 months). The actual relevance of this mechanism in the environment, particularly over longer timescales, is unknown.

3.3.2.2 Mammals

Several studies have investigated the uptake of decaBDE in mammals, but have not specifically considered metabolites (e.g. Norris et al., 1973 and 1974; El Dareer et al., 1987; Viberg et al., 2001 [ABST]; Thomas et al., 2003 [ABST] and 2005; Richardson et al., 2007

[ABST]; Huwe et al., 2008a and 2008b; Hardy et al., 2009; Biesemeier et al., 2011). In contrast to studies with Brown Rats (*Rattus norvegicus*), in which decaBDE has low systemic bioavailability and is mainly associated with blood-rich tissues, studies with Grey Seals (*Halichoerus grypus*) suggest more extensive uptake and show that significant concentrations may accumulate in blubber.

The following studies considered the products of metabolism:

i) Mörck and Klasson Wehler (2001) [ABST] investigated the metabolism of ^{14}C-labelled decaBDE (purity not given) using conventional and bile duct-cannulated Brown Rats (*Rattus norvegicus*). The rats were given a single oral dose of 3 µmole/kg (~2.9 mg/kg) of the test material suspended in a mixture of Lutrol F127, soya phospholipid and water. Excreta were collected over the following 72 hours and analysed for ^{14}C content and phenolic metabolites. The results of the study showed that the major route of excretion (~90% of the dose within 3 days) was via the faeces. Detailed analysis of the faeces showed that 22%, 42% and 45% of the radioactivity present at day 1, 2 and 3 respectively was present as phenolic metabolites. In all, eight phenolic metabolites were identified as their corresponding methyl derivatives. These were dimethoxylated derivatives of penta- to octaBDEs (the dihydroxyl groups were always on the same ring). The remaining radioactivity present in the faeces was identified as unchanged decaBDE.

ii) Mörck et al. (2003) performed a study designed to identify the metabolites of decaBDE in rats. Absorption was maximised by careful choice of test vehicle (several different solvents were investigated for this purpose). In the experiment, eight male Harlan Sprague-Dawley rats were given a single oral dose of ^{14}C-labelled decaBDE solution by gavage and urine and faeces were collected at 24-hour intervals for three days (urine) or 7 days (faeces). Four rats were sacrificed after 3 days and the remaining four rats after 7 days. Around 65% of the dose excreted in faeces was as metabolites (phenolic compounds with between five to seven bromine atoms/molecule, and a small amount (corresponding to <0.5% of the initial dose) of three nonaBDEs, which were not present in the test substance administered to the rats). It was postulated that metabolism occurred in the liver and small intestine and that a reactive metabolite (e.g. an arene oxide or a catechol) may be involved in later metabolic steps (debromination of decaBDE was thought to occur as a first step). (See ECB, 2004 & 2007 for a more detailed summary.)

iii) Sandholm et al. (2003) investigated the bioavailability and half-life of decaBDE in the blood of eighteen male Sprague-Dawley rats dosed by gavage (1.92 mg/kg), and eighteen dosed intravenously. Blood samples were collected at intervals up to 144 hours after administering the doses (6 days). Similar metabolic profiles were obtained from both groups of animals. The neutral fraction was dominated by unchanged decaBDE, but traces of three nonaBDEs were also found. The phenolic fraction was found to contain at least thirteen metabolites containing bromine, but only three were present in high enough concentration to allow tentative identification. Monohydroxylated nonaBDE and monohydroxylated octaBDE were found to be present. The third metabolite was not unambiguously identified.

Analysis of the plasma samples from the Mörck et al. (2003) metabolism study showed that the level of radioactivity present in the phenolic fraction was around 4 times higher than in the neutral fraction at both days 3 and days 7. The neutral fraction was again found to contain mainly unchanged decaBDE, along with traces (<0.5% of the total peak area) of three nonaBDEs. However, it was not possible to determine the nature of the metabolites in the phenolic fraction.

The authors speculated that a possible explanation for the high concentrations of metabolites relative to the parent compound found in plasma at day 3 could be a result of reversible binding of the metabolites to the thyroxine hormone transporting protein transthyretin. DecaBDE might undergo first pass metabolism (in the GI-tract) before reaching the circulatory system. (See ECB, 2004 & 2007 for a fuller summary.)

iv) Huwe (2005) [ABST] and Huwe and Smith (2007) studied the uptake and accumulation of decaBDE in rats exposed via their diet. (This study was not summarised in previous EU risk assessment reports.) Sprague-Dawley rats (80-day old) were trained to eat a diet consisting of 12 g ground rat chow topped with 200 µl of corn oil over a one hour period each morning (this method of dosing a restricted diet ensured that the dose was completely consumed by the rats and also minimised the impact of body mass changes during the study). No traces of hepta- to decaBDE were evident in the food or corn oil used in the study. The decaBDE used in the experiment had a purity of 98.5% and was dissolved in corn oil and a small amount of toluene. Other PBDEs that were found to be present in the decaBDE used included nonaBDEs, octaBDEs and a trace of one heptaBDE (BDE-183). The solution was stirred overnight under a stream of nitrogen to allow the toluene to evaporate, giving a final concentration of 18.9 µg decaBDE/ml oil. At the start of the test the decaBDE solution was added to the diet of eighteen rats each day for 21 days at a concentration of 0.3 mg/kg food (each rat received a daily dose of 3.8 µg). Eight control rats received the same diet without the addition of decaBDE.

At various times after the last dose was administered (starting 24 hours after the last feeding up to 21 days after last feeding) groups of three dosed rats were killed and the amounts of PBDEs in the various tissues and organs were determined. Low amounts of decaBDE and nonaBDE were also found to be present in the control rats but the tissue levels were 10 to 20 times lower than in the exposed rats. In addition, some PBDEs were also found to be present in laboratory blanks. The data were corrected for the levels found in the control rats.

The concentration of decaBDE in dosed rats was found to be around two to three times higher in the liver than the carcass (the lipid contents were similar at 4.13% for the liver and 4.32% for the carcass). This is consistent with the uptake of decaBDE being associated with blood proteins rather than lipid fractions.

The amounts of several lower PBDE congeners present in the rats after 21 days' exposure were higher than could be accounted for by the dose given. This included two octaBDEs (BDE-197 and BDE-201) and one nonaBDE (BDE-207)[58]. The amounts of these congeners present in the tissues were around 155% (BDE-207), 845% (BDE-201) and 1,170% (BDE-197) of the administered dose and suggest that they were formed by (meta-) debromination[59]. These substances accounted for a relatively small fraction of the total decaBDE dose (<3%). The concentration of the two octaBDE congeners (BDE-197 and BDE-201) continued to increase during the 21-day depuration phase of the study. This provided further support for their formation in the rats by metabolism.

Evidence for more extensive metabolism came from mass balance considerations. The amount of unchanged decaBDE retained in the tissue and plasma after 21 days' exposure was around 5%, with the amount in faeces being around 50% of the dose. As the debromination to lower PBDE congeners was estimated to be <3%, this left around 42% of the dose unaccounted for, possibly as non-extractable bound residues or as unknown

[58] The absence of analytical standards for some congeners at the time of the analysis does not affect the findings, because the same analytical uncertainties would apply equally to the analysis of the amounts present in the food samples and the rats (i.e. although the *absolute* concentrations may be uncertain, the *relative* concentrations between the food and rats would not be affected by the lack of standards).

[59] Another possible explanation could be degradation of decaBDE during the tissue handling, extraction and analytical procedures. Precautions were taken to minimise exposure to light during the sample preparation and analysis during the study. To investigate further, a control carcass was spiked with decaBDE at similar concentrations to those found in the dosed animals. These spiked control samples (carried out in triplicate) were processed using the same analytical methodology as the dosed animals and this found that the concentrations of BDE-197, BDE-201 and BDE-207 were approximately ten times lower than found in the dosed animals. Formation during the analytical procedure therefore could not, on its own, account for the levels of these congeners found in the dosed animals. Furthermore, the pattern of octaBDEs and nonaBDE found in the spiked samples was different from that found in the dosed animals.

metabolites. No further analysis for possible bound or hydroxylated metabolites was carried out[60].

v) Riu et al. (2006 [ABST] and 2008) investigated the disposition and metabolism of ^{14}C-labelled decaBDE in pregnant Wistar rats. (The abstract was summarised in previous EU risk assessment reports.) The test substance had a radiochemical purity of >99.8%. Three rats were used in the study (mean bodyweight 284 g). The animals were force fed a daily dose of the ^{14}C-decaBDE dissolved in peanut oil from gestational days 16 to 19 (the dose rate was 2.0 mg/kg bodyweight/day). During the study the rats were allowed free access to water and a standard diet, and urine and faeces were collected daily. The animals were killed on day 20 of gestation (24 hours after the last dose of ^{14}C-decaBDE) and the amounts of ^{14}C-label, parent compound and metabolites were determined in various organs and tissues.

The total radioactivity recovered in the experiment was 91.1% of that dosed. A detailed analysis of ^{14}C-metabolites was carried out using HPLC with a radioactivity detector. Around 97% of the radiolabel recovered from the organic fractions of the faeces was found to be unchanged decaBDE. However, three ^{14}C-labelled metabolites that were more polar than decaBDE were also evident. Similarly, unchanged decaBDE accounted for 96.4%, 86.4% and 91.9% of the radiolabel in the organic fractions of the stomach, small intestine and large intestine respectively. Metabolites with a higher polarity than decaBDE were also evident in the aqueous fractions from the stomach, small intestine, large intestine and faeces accounting for around 34.4%, 70.5%, 50.6% and 17.5% respectively of the radiolabel present in the aqueous fractions. For urine, the majority of the radiolabel present was found to be as more polar metabolites and no unchanged decaBDE was evident.

For plasma and other tissues, the proportion of metabolites present ranged from around 9% in adrenals, ovaries and liver to around 30% in the carcass. The remaining radiolabel was generally attributable to unchanged decaBDE although a small amount of non-extractable radiolabel was evident in some tissues.

Several main metabolites were isolated from faeces and tissues and identified using mass spectrometric methods. These were nonaBDEs (BDE-206, BDE-207 and BDE-208), an unidentified octaBDE and a hydroxylated octaBDE derivative. Checks were carried out to determine whether degradation of decaBDE occurred during the preparation and administration of the dosage solution or during the analytical procedure. This showed that no degradation occurred meaning that the metabolic products were formed in the animals.

Overall, the metabolites were estimated to account for around 7% of the total radioactivity administered. The result showed that at least 19% of the administered dose was absorbed and recovered in the body tissues plus carcass. Excretion via faeces was the main elimination route. Metabolism of decaBDE was likely to be occurring in the rats, with the initial formation of nonaBDEs (BDE-206, BDE-207 and BDE-208), although it is possible that such reductive debromination could have occurred in the gut by the microflora present. The liver was found to be a target tissue for decaBDE (6.5% of the total radioactivity and a concentration of 11 mg/kg) but the highest concentrations were found to be present in certain endocrine glands (e.g. adrenals and ovaries). The amount of radiolabel found to cross the blood-brain barrier was relatively small but 0.5% of the dose

[60] Malmberg et al. (2004 [ABST] and 2005) studied the formation and retention of hydroxylated PBDE metabolites in rat blood. A group of ten rats were given a single intraperitoneal dose of an equimolar (3 µmol/kg body weight for each substance) mixture of 2,2'4,4'-tetraBDE, 2,2',4,4'5-pentaBDE, 2,2',4,4',6-pentaBDE, 2,2',4,4'5,5'-hexaBDE, 2,2',4,4',5,6'-hexaBDE, 2,2',3,4,4',5',6-heptaBDE and decaBDE. The mean concentration of decaBDE in plasma was found to be 1,200 pmol/g fresh weight 24 hours after exposure and this fell to 60 pmol/g fresh weight five days after exposure. A total of sixteen hydroxylated (three of which were dominant) and two dihydroxylated PBDE metabolites were identified in the plasma samples. As the rats were exposed to a mixture of PBDEs it is not possible to distinguish which metabolites, if any, originated from decaBDE.

was found in foetuses indicating that decaBDE or metabolites can cross the placental barrier.

vi) An *in vitro* metabolism study with decaBDE using rat microsomes has been carried out by Mas et al. (2008b) [ABST]. (This study was not summarised in previous EU risk assessment reports.) The tests were carried out using phenobarbital-, β-naphthoflavone- and clofibrate-treated rat liver microsomes. The decaBDE used in the study had a purity of 98%. Stock solutions of the test substance were prepared in dimethyl sulphoxide (concentration 140 mg/l). DecaBDE was thought to be soluble in dimethyl sulphoxide at this concentration.

The metabolism tests were carried out by pre-incubating 1.5 µM of decaBDE (in dimethyl sulphoxide) with 1 mg/ml of the hepatic microsomes in a 0.1 M buffer (pH 7.5) for five minutes with shaking at 37°C. After preincubation the reaction was initiated by the addition of a nicotinamide adenine dinucleotide phosphate (NADPH) regenerating system and metabolism was stopped after 30, 60 and 120 minutes incubation (the total volume of the system was 1 ml). Control incubations were carried out in the same way, except sodium bicarbonate was added instead of the NADPH regenerating system.

The control incubations did not show any decrease in the concentration of decaBDE present, showing that there was no significant abiotic degradation of decaBDE under the conditions used. Loss (or metabolism) of decaBDE was evident in the hepatic microsomes, with around a 31% to 58% decrease in concentration being evident after 30 minutes in the different systems used. The highest loss was found with the phenobarbital-treated system. No further decrease in the decaBDE concentration was evident in any of the systems between 30 minutes and 120 minutes incubation time.

Chemical analysis did not find evidence of any lower molecular weight PBDE congeners as transformation products. The study also investigated the formation of hydroxylated derivatives (with between two and four bromine atoms per molecule) but again there was no evidence for the formation of such derivatives with this level of bromination.

vii) McKinney et al. (2011) assessed the biotransformation of PBDEs using an *in vitro* system based on liver microsomes prepared from Polar Bear (*Ursus maritimus*), Beluga Whale (*Delphinapterus leucas*), Ringed Seal (*Pusa hispida*) and Brown Rat (*Rattus norvegicus*). (This study was not summarised in previous EU risk assessment reports.) Microsomes were incubated with either decaBDE alone or a mixture of brominated flame retardants (decaBDE, decabromodiphenyl ethane, BDE-99, -100 and -154) in a buffer with an internal standard. Control tubes contained the substance/mixture spike and buffer. Sample tubes contained the same, but also contained 10 mM dithiothreitol and nicotinamide adenine dinucleotide phosphate regenerating system. External standards were prepared containing the appropriate substance/mixture solution to monitor recoveries. Blanks containing buffer only, or buffer and microsomes were also run. One milligram of microsomal protein was added to each sample and control tube (enzyme activity in the controls was prevented by the addition of methanol).

Following completion of the assay, separation and quantification of all neutral brominated analytes was performed by gas chromatography-mass spectrometry (GC-MS). Extent of depletion was reported as the fraction of the substance remaining in the samples compared to the controls after the assay, using internal standard normalisation. Some of the final extracts from the individual assays were then derivatised to additionally investigate the possibility of oxidative metabolite formation. Derivatised fractions were also analyzed for methoxylated substances.

The assays were designed to optimize multiple enzyme systems, so that both reductive and oxidative metabolic pathways would be observed. This was felt to be important for decaBDE, since the phase I cytochrome P450 mediated direct hydroxy group insertion or arene-epoxide formation on a fully halogenated aromatic compound was considered unlikely as an initial metabolic step. Greater depletion of decaBDE (14-25% of 30 pmol)

occurred in individuals from all species relative to depletion of lower molecular weight PBDEs (i.e. BDE-99, -100, and -154; 0-3% of 30 pmol). However, no evidence of simple debromination was observed (other than possible formation of nonaBDEs) and metabolite concentrations were also low to non-detectable, despite substantial parent depletion. The authors proposed a number of explanations for the lack of metabolite detection (e.g. reactive metabolites covalently bound to proteins and/or lipids, a variety of PBDE or hydroxylated metabolites formed at concentrations below the limit of detection, and highly brominated phenolic metabolites (which might not have been effectively derivatized by the analytical method). A low recovery of conjugated, water-soluble metabolites that would have remained in the aqueous phase on extraction with hexane could also be an explanation, although it was considered unlikely. However, in a response to the paper, Hardy (2012) argued that the apparent depletion of decaBDE might have been due to its low solubility and tendency for non-specific binding to surfaces and particulates. Low solubility compounds are not properly assayed by many *in vitro* systems due to their precipitation and/or adherence to the walls of the vessel, which eliminates interaction with microsomal enzymes. The authors suggested that future studies should use radio-labelled substance to improve the ability to track loss of the parent compound.

viii) PBDE levels have been investigated in domestic cats (*Felis catus*) and their food (Venier et al. (2007) [ABST], Dye et al. (2007a) [ABST] and Dye et al. (2007b); the latter reference gives further details of the levels of decaBDE found). (This study was not summarised in previous EU risk assessment reports.) Serum samples (n = 23) were obtained from veterinary teaching hospitals in North Carolina, Massachusetts and Georgia, United States. Twelve samples of dry cat food and 24 samples of canned cat food were obtained from shops in Indiana. The quality assurance and quality control procedures included the routine analysis of procedural blanks (the levels of PBDEs in the blank samples were low and so it was not necessary to correct for this). Although the amount of one nonaBDE (BDE-207) accounted for only 1-3% of the total PBDEs in dry food, the levels in serum accounted for around 17% of the total PBDEs in dry-food-eating cats (the serum levels were displayed graphically only). Similarly, the BDE-207:decaBDE ratio in dry food was approximately 0.03 but the ratio found in serum was fairly constant across all cats (0.51, 0.54 and 0.63 in young, sick (non-hyperthyroid) and hyperthyroid cats respectively). The authors suggested that these findings indicate that BDE-207 is either much more accumulative than decaBDE or that decaBDE is metabolised to BDE-207 in cats (or a combination of the two). However, the contribution of other sources (e.g. household dust) to the measured serum concentrations is unknown, and it is possible that the food concentrations are not representative of the food that the cats actually ate. This study therefore only provides weak evidence of transformation in this species.

ix) The fate of decaBDE in lactating domestic cows (*Bos primigenius*) has been studied by Kierkegaard et al. (2007). The study was carried out over a three month (thirteen week) period at an experimental husbandry farm in Devon, UK. The study was originally designed to determine the long-term mass balance of polychlorinated biphenyls in the cows (Thomas et al., 1999) but the stored samples (frozen and stored in the dark) from two cows from the original study were re-analysed for hepta- to decaBDE congeners. The feed used in the study consisted of silage, concentrate and mineral supplement. The silage was produced on-site and was stored tightly-covered. Three batches of silage were used during the study (the decaBDE content of each batch was determined). The food (silage, concentrate and mineral supplement) was not deliberately spiked with PBDEs for the study, so the levels present were those in the feed as received.

The cows were kept indoors and allowed unlimited access to silage. Feed consumption and milk production were measured daily. Sub-samples of milk and faeces from both cows were collected once per week for 13 weeks from bulk morning and evening samples. These sub-samples were pooled into five samples (three representing a three week period and two representing a two week period) prior to analysis. At the end of the thirteen week period one of the cows was slaughtered and samples of adipose tissue from six lipid

compartments (omental fat, ventral abdominal fat, lumbar fat, dorsal thoracic fat, kidney fat and heart fat), kidney, heart and leg muscle were collected and analysed.

The limit of quantification of the analytical method used was in the range 0.4-150 ng/kg lipid weight (or dry matter). Procedural blanks covering the whole extraction and analytical procedure were run in parallel with the samples. It was found the decaBDE was subject to a small amount of degradation during the extraction/sample clean up and analytical detection. The degradation products included nonaBDEs. Although the amount of decaBDE degraded was not significant in terms of quantifying the amounts of decaBDE (the extent of degradation was estimated at around 4% of the total decaBDE present), it was estimated that this degradation of decaBDE could have accounted for up to around 50% of the amount of the individual nonaBDEs determined in some samples. Therefore the results of the analysis for nona- and octaBDEs were corrected for this degradation.

Silage was found to be the main source of PBDEs in the diet. The major output route from the cows was via faeces. The concentrations of PBDEs in milk were generally low (the contribution of this source to the total output of decaBDE was estimated at <1%).

When the concentrations were converted into mass flows, highly variable estimates for the input rate of each congener were obtained. This resulted mainly from the different amounts of PBDEs found in the three batches of silage used in the study (for example the levels of decaBDE in the second batch were 20-30 times higher than found in the first and third batch). A concurrent increase in the output flow (milk plus faeces) was observed in both cows during feeding with the second batch, but this increase was only by a factor of four compared with feeding with the first and third batch. As a consequence of this, the calculated output rate exceeded the calculated input rate at the beginning and end of the experiment, whereas the opposite was true in the middle period of the experiment.

Overall it was estimated that over 90% of the PBDE body burden was contained in the adipose tissue (it was further estimated that adipose tissue accounted for around 15% of the live weight of the cows).

The congener profile present in the various samples was investigated for evidence of possible biotransformation of decaBDE to lower PBDE congeners. This analysis suggested that four congeners (2,2’,3,3’,4,4’,5,6,6’-nonaBDE, 2,2’,3,3’,4,4’,5,6’-octaBDE, 2,2’,3,3’,4,4’,6,6’-octaBDE and possibly 2,2’,3,4,4’,5,6’-heptaBDE) were present in lipids at higher concentrations than might be expected based on their concentration in the feed. Several possibilities were put forward by the authors to explain this, including differences in dietary absorption between congeners or biotransformation in the digestive system. Other possibilities such as photochemical degradation of decaBDE during sample handling and debromination occurring in the rumen were ruled out because all samples were stored in the dark and degradation during sampling/analysis was accounted for in the analytical approach used and no differences were seen in the congener profiles between faeces and feed (a difference would be expected if debromination in the rumen was occurring).

Discussion

Oral and dietary exposure studies with Brown Rats show that decaBDE can be metabolised to nona- and octaBDEs, which account for a small fraction of the total absorbed dose (e.g. <3% after 21 days, although the levels were found to increase during depuration). A significant amount of the absorbed dose (~40-50%) might be transformed to mono- and di-hydroxylated PBDE metabolites or non-extractable bound residues, the properties of which are unknown. The studies are generally of short duration (from a few days to a few weeks), so the potential for more extensive transformation over longer time scales following repeat exposure is unknown.

In vitro studies using tissues from Polar Bear, Beluga Whale and Ringed Seal (McKinney et al., 2011) suggest that decaBDE might be metabolised to as yet unknown transformation products in these species (along with possible formation of nonaBDEs).

There is weak evidence of possible transformation to nonaBDEs in domestic cats from a monitoring study (other explanations for the observations are possible). The only other species in which metabolism has been investigated is the domestic cow. A retrospective analysis of animals exposed via silage over three months suggested that there might have been some transformation to octa- and even heptaBDEs, but accumulation from the diet could not be ruled out.

The uptake of decaBDE may be relatively high in some species (e.g. seals), so the lack of comparative metabolic data makes it difficult to draw general conclusions from these data.

3.3.3 Summary and discussion of transformation in biota

Fish can take up decaBDE from their diet, and transform it into at least hexa- and heptaBDEs, as well as methoxylated and hydroyxlated PBDEs. The yield of the hepta- and hexaBDE metabolites is generally low (typically below 5% of the absorbed decaBDE dose in the various studies) and the actual amounts are small, but the formation of precursors is more extensive and these could provide an ongoing source over longer time periods. Species differences in the extent of transformation are apparent, and further studies implicate the role of enzymes involved in thyroid hormone homeostasis with limited oxidative, cytochrome P450-mediated metabolism. Deidoinase enzymes convert T4 to T3 by removing iodine from the *meta*-position of the diphenyl ether backbone. The same pattern is observed for the reductive debromination of decaBDE in fish species.

Steady state may take several months to achieve under constant exposure situations. The most persuasive evidence of the relevance of this process in the environment is from mesocosm studies that show that a single decaBDE application of 2.3 g to an aquatic system volume of 180 m^3 gives rise to at least 5-10 µg/kg dw of tetra- to heptaBDEs in fish after three months. Modelling suggests that further accumulation might occur over the life time of the fish.

Birds might be able to debrominate decaBDE to at least octaBDEs, based on limited data. The actual relevance of this mechanism in the environment, particularly over long timescales, is unknown. It is possible that birds will form other metabolites as well, but no information is available.

Oral and dietary exposure studies show that rats can metabolise decaBDE to nona- and octaBDEs, which account for a small fraction of the total absorbed dose (e.g. <3% after 21 days). A significant amount of the dose might be transformed to hydroxylated metabolites or non-extractable bound residues. The studies are generally of short duration (from a few days to a few weeks), so the potential for more extensive transformation over longer time scales following repeat exposure is unknown. There are no conclusive metabolism data for other mammalian species, although *in vitro* studies suggest a similar metabolite profile in bears, whales and seals.

The total amount of decaBDE residing in biota following release is difficult to estimate, and will be significantly lower than that residing in sediments and soils. Nevertheless, the high persistence of decaBDE in these media means that organisms may be exposed continuously. Although the level of uptake might be relatively low in some species, it is higher in others and the available monitoring data show that both aquatic and terrestrial organisms of many species are contaminated with the substance. Although decaBDE appears to associate more with blood-rich tissues, it can also be stored in adipose tissue in some species. There is also evidence to suggest that the extent of metabolism of decaBDE may vary significantly between species. It is therefore possible that the formation of small percentages of hexa- to heptaBDE congeners by metabolic processes over long time scales could be important in some cases (for example, in fish or organisms that undergo significant changes in adipose stores during their life cycle, e.g. during migration or hibernation). This will add to the organism's body burden

from exposure to these substances from legacy sources and transformation of decaBDE in sediment and soil.

3.4 Secondary poisoning

Not relevant for this dossier.

4 Human health hazard assessment

This dossier has been prepared to identify decaBDE as an SVHC on the basis of its PBT/vPvB properties. A human health hazard assessment is not relevant for this dossier.

5 Environmental hazard assessment

Aquatic toxicity data for decaBDE are relevant to this dossier in the context of the toxicity profile of the PBDE family as a whole (see Appendix 1). The registration dossiers provide study summaries for several ecotoxicity end points that were submitted under the ESR. Detailed reviews of these studies and additional data were included in EC (2002), ECB (2004 and 2007a) and EA (2009). Despite the limited database, it was considered unlikely that significant acute or chronic toxic effects would occur in aquatic organisms at concentrations up to the water solubility limit (by analogy with commercial octaBDE products). No significant adverse effects were observed in sediment or soil organism toxicity tests. (No toxicity data were available for birds.)

Since the EU risk assessment reports were completed, some additional toxicity data have become available in the open literature (these have not been summarised in the registration dossiers).

Aquatic toxicity

1) Noyes & Stapleton (2010) [ABST] and Noyes et al. (2011) investigated decaBDE accumulation and effects in Fathead Minnows (*Pimephales promelas*) following dietary exposure (see Section 3.3.1.2). As part of this study, thyroid and liver tissue morphologies were examined in samples of juvenile fish that had been exposed to decaBDE in their diet for 28 days at a concentration of 9.8 ± 0.16 µg/g food. Histological examination showed significantly increased thyroid follicular epithelial cell heights and vacuolated hepatocyte nuclei compared to control fish. Combined with evidence of effects on deiodinase activity, the authors suggested that juvenile fish may be susceptible to thyroid disruption by decaBDE.

2) Kuo et al. (2010b) fed juvenile Lake Whitefish (*Coregonus clupeaformis*) a diet containing decaBDE at four nominal concentrations (control, 0.1, 1, and 2 µg/g diet) for 30 days (see Section 3.3.1.2). Otolith increment widths were narrower in fish from the highest diet concentration administered, suggesting that the treatment may have affected growth rates. The historical variability of this end point is unknown, and the exposure duration was relatively short, so the significance of this finding is unclear.

3) He et al. (2011) exposed Zebrafish (*Danio rerio*) embryos to decaBDE solutions (ranging from 0.001 to 1 µM, or 0.96 – 960 µg/l) in five treatment groups (including a solvent control) for 150 days (5 months), using a semi-static exposure regime. The test substance purity was >97% w/w. The mature fish were also used to produce an F1 generation, without decaBDE exposure. Test concentrations were not confirmed by

chemical analysis. DecaBDE exposure affected overall fitness (measured by condition factor), gonad development, male gamete quantity and quality in F0 parental fish. For F1 offspring without exposure, the parental treatment led to delayed hatch and motor neuron development, loose muscle fibre, slow locomotion behaviour in normal conditions, and hyperactivity when subjected to light–dark photoperiod stimulation. Significant differences from the control were observed at the lowest dose. It therefore appears that chronic low dose decaBDE exposure not only affects F0 growth and reproduction at concentrations at or around 0.96 µg/l, but also elicits neurobehavioral alterations in F1 offspring that were not exposed (suggesting a transgenerational effect). The lack of chemical analysis means that it is not possible to establish the actual exposure concentrations with confidence. In addition, the study does not appear to have followed any standard test protocol for fish life cycle tests. Its reliability therefore remains uncertain, but the findings are a cause for concern.

4) Chen et al. (2012) investigated the effects of decaBDE on Zebrafish (*Danio rerio*) embryos. (This study was not summarised in previous EU risk assessment reports). The test substance was >98% pure (laboratory sourced with no details of impurities). The embryos (two hours post-fertilization) were exposed to nominal decaBDE concentrations of 0 (solvent control), 0.008, 0.38 and 1.92 mg/l for 14 days using a semi-static regime (daily renewal). Larvae were fed twice a day. Three replicates per concentration were used with (reportedly) 400 embryos per replicate. Solutions were prepared using dimethyl sulfoxide (0.01% v/v), and all nominal concentrations exceeded the reported solubility of decaBDE in pure water (by a factor of ~100 to 10,000). It is not known what concentrations the fish were actually exposed to in practice.

   No effects on hatching or malformation were observed in any treatment. Statistically significant effects on weight and survival were seen at the highest concentration (e.g. 32% mortality was observed compared to 27% in the controls). Deca-, nona- and octaBDE congeners were detected in fish at the end of the test, with concentration dependent levels found. For the top dose 38 µg/g of decaBDE was detected in fish.

   Changes in the transcription of several genes that affect thyroid hormones were observed as well as effects on thyroid hormones themselves with T3 (iodothyronine) concentrations increasing and T4 (thryroxine) levels decreasing. The effects were statistically significant and concentration dependent for T3 and T4, and statistically significant and generally concentration dependent for the twelve genes examined.

   The test is not valid given the high nominal test concentrations above the water solubility limit. However, it provides some indication that decaBDE has the potential to cause adverse effects in Zebrafish early life stages, with impacts on T3 and T4 concentrations.

5) Qin et al. (2010) exposed African Clawed Frog *Xenopus laevis* tadpoles to a commercial decaBDE product (purity 98.5% w/w) at nominal concentrations of 1 – 1,000 ng/l in water. Test substance exposure of tadpoles was initiated at stage 46/47 (free swimming larvae on the fifth day post-fertilization, system of Nieuwkoop and Faber). Test solutions were completely replaced twice weekly. After forelimb emergence (FLE, stage 57/58), cumulative percentage of FLE (an endpoint for evaluating metamorphosis time) was recorded in each treatment. Exposure was terminated at stage 62 (metamorphic climax). At this point, twelve tadpoles from each treatment were randomly chosen to examine thyroid gland histology and TR mRNA expression in tail tissues.

   Total PBDE concentrations in the test media (sum of 39 congeners using gas chromatography-mass spectrometry using negative chemical ionization (NCI) in the selected ion monitoring mode) were monitored at day 1–4 in each exposure group. The PBDE burden was also measured in three tadpoles from each tank at the end of the experiment.

The mean measured concentration of total PBDEs (two samples per time point per treatment) was found to decline by 77.5% in the highest treatment, and between 77.2 and 90.9% in the lower treatments, over the first four days of the experiment. Therefore although the test solutions were replaced periodically, the tadpoles were not exposed to a constant dissolved concentration. It is possible that at least some of the 'missing' substance was adsorbed to the food, and tadpole body burden was found to be dose-related (with a mean total PBDE concentration of 1,400 ± 129 ng/g ww in the highest treatment). The PBDE burden was dominated by BDE-209 (96 – 99%), and small amounts of lower molecular weight PBDE congeners (such as BDE-47, -85, -99, -153, -154 and -183) were also detected in the tadpoles, while BDE-205 and -206 were not detected. PBDEs were not detected in control tadpoles.

No malformations or abnormal behaviour were observed in any treatment. Besides natural mortality, mechanical damage incurred during transfer of tadpoles from the hatching tank to exposure tanks and during water exchange led to low survival. Nevertheless, the survival rate (tadpoles reaching stage 62 among 35 tadpoles from each tank) was not significantly different (at $p < 0.05$) between treatments and the solvent control (72.9 ± 2.0%). The first tadpoles displaying FLE were observed on exposure day 27 in the solvent control group (two tadpoles) and in the nominal 10 ng/l treatment group (one tadpole). The onset of FLE occurred on day 29 in all the other treatment groups (four tadpoles at nominal 1 ng/l, three tadpoles at nominal 100 ng/l, and one tadpole at nominal 1,000 ng/l). Compared with the solvent control, the time to FLE (indicated by cumulative percentage of FLE) was delayed in all decaBDE treatments, and this was statistically significant at the highest concentration. The overall magnitude of the delay at the highest concentration was about 10 days (read from a graph).

Histological examination showed that decaBDE at all tested concentrations caused histological alterations in the thyroid gland compared with the solvent control (multilayer follicular epithelial cells). No obvious concentration dependence was observed, except for markedly increased follicle size accompanied by partial colloid depletion and an increase in the peripheral colloid vacuolation at the highest test concentration.

All tested concentrations induced a down-regulation of thyroid receptor mRNA expression in tail tissue compared with the solvent control group, with no statistical difference between the three highest test concentrations.

This study therefore suggests that decaBDE can delay metamorphosis of *X. laevis* tadpoles, with a tentative no-observed effect concentration (NOEC) below 1,000 ng/l (0.001 mg/l). This appears to be accompanied by histological changes in the thyroid gland and thyroid receptor interactions. Since the study was not performed in accordance with standard test guidelines or GLP, it might be premature to draw a final conclusion on this.

Amphibian metamorphic development is controlled by thyroid hormones through the regulation of target gene transcription via interaction with thyroid receptors. The observations in the *Xenopus* study might therefore be related to thyroid interactions. Several studies have investigated thyroid effects of decaBDE in mammals, but these are not summarised here (see EFSA (2011) for a recent review). The following additional data are available for aquatic species:

- Potential thyroid hormone disruption in fish by several PBDEs was investigated *in vitro* by Morgado et al. (2007). The study used a competitive binding assay developed using sea bream recombinant transthyretin (TTR). 50 ng of TTR were incubated in 200 µl of buffer containing 0.1 nM [^{125}I]-3,5,3'-L-triisdothyronine (T_3) in the presence of increasing amounts of unlabelled T_3 or PBDEs (concentration 0-10 µM) for 2 hours on ice. Neither decaBDE nor BDE-206 (or indeed several hexa- to octaBDEs) showed any binding to TTR, as shown by their inability to displace [^{125}I]-T_3. The influence of metabolism appears not to have been considered in this study.

- Schriks et al. (2006) studied the potential for a nonaBDE (BDE-206) to cause thyroid hormone-mediated effects in the amphibian *X. laevis*. The effects of the test substance on tail tip regression caused by exposure to 3,3',5-triiodo-L-thyronine (T_3) were investigated in organ culture (*ex vivo*). Tail tips were exposed to the test substance both in the presence (20 nM which is equivalent to the EC_{50} for T_3) or absence of T_3. The T_3-induced tail tip regression was found to be antagonised by exposure to BDE-206 in a concentration-dependent manner at concentrations from 1 to 1,000 nM (1 - 960 µg/l) after 4 days, although the effect was only observed at the two highest doses after 6 days. These concentrations are likely to be above the water solubility of the substance. No effects on tail regression were seen with the exposure without T_3. (It is assumed that this is the same study as that briefly reported by Murk et al., 2007 [ABST].)

In summary, the lowest aquatic NOEC appears to be around 0.001 mg/l (1 µg/l). This is in the region of the reported solubility limit in pure water. Due to methodological limitations imposed by this poorly soluble substance, repeat studies conducted according to recognised protocols (at or below the solubility in the test media) would be needed to confirm the results. However, these new data raise concerns for toxicological effects that had previously been discounted.

Avian toxicity

Sifleet (2009) injected decaBDE into the yolk sac of chicken (*Gallus gallus*) eggs. (This study was not summarised in previous EU risk assessment reports). A >98% purity laboratory source of decaBDE was used (impurities not specified). Two experiments were reported, one using a single nominal concentration and the other using three doses. These were not concurrent, but run in consecutive months. In both studies an emulsion vehicle was used to introduce the test material into egg.

For the single dose study a total of 198 eggs were used as follows: un-injected eggs (39), vehicle injected eggs (80) and decaBDE injected eggs (79), at 80 µg/egg using an injection volume of 100 µL. For the second experiment 219 eggs were used as follows: un-injected eggs (30), vehicle injected eggs (43), decaBDE injected eggs at 40 µg/egg (47), 20 µg/egg (49) and 5 µg/egg (50), this time using an injection volume of 50 µL. No replicates were run.

Mortality was seen in the vehicle injected (control) eggs, with greater mortality seen for the higher injection volume (16%) than the lower volume (9%).

For the single dose study, 98% mortality was observed with 77 of the 79 decaBDE-treated eggs dying following injection. This was confirmed to be statistically significant compared to the vehicle-injected eggs. In the second experiment, statistically significant differences in mortality were seen in the medium and higher doses compared to the vehicle injected eggs.

The results of the two experiments were then combined to calculate an LD_{50} of 44 µg/egg (740 µg/kg ww) over a 20-day period following injection. The statistical models used to detetmine the LD_{50} included a term to account for the mortality due to the emulsion carrier (i.e. it was subtracted out of the LD_{50} value). The mortality rate from exposure to the emulsion vehicle was estimated to range between 16-18%.

Additional analysis was conducted for the 20 µg/egg dose to examine the distribution in five compartments (yolk, liver, brain, heart, and carcass). This indicated that decaBDE is transported by the blood throughout the embryo, although 80% of the dose remained in the yolk sac. No definitive evidence of metabolic debromination by chicken embryos was seen. Both tissue extracts and the dosing emulsion exhibited low levels of nonaBDE congeners, with the highest nonaBDE concentrations found in the yolk.

This study did not conform to any standard protocol. Yolk injection allowed the preservation of a closed system within the egg, but it is likely that the test substance was not dispersed evenly throughout the entire yolk (the emulsion may have spread somewhat from the original

"bubble" but would have stayed near the top of the yolk, readily accessible to the embryo). This does not replicate the distribution that would have occurred following transfer from the parent to its egg. In particular, the embryos would be exposed to higher concentrations than if the substance was dispersed throughout the yolk, with greater exposure as the bubble rotated with the embryo inside the eggs throughout incubation. The results should therefore be viewed with caution. However, they suggest that decaBDE concentrations typically found in bird eggs in the wild (in the range 1-100 µg/kg ww, but up to about 420 µg/kg ww) are around a factor of 2-10 times lower than a level that may induce significant mortality. Such a margin is not high. It does not take account of potential sub-lethal effects, and the author noted that additional decaBDE would likely have been assimilated following hatching and resorption of the remaining yolk.

6 Conclusions on the SVHC Properties

6.1 PBT, vPvB assessment

6.1.1 Assessment of PBT/vPvB properties – comparison with the criteria of Annex XIII

DecaBDE is a very persistent substance in terms of the Annex XIII criteria, as demonstrated by simulation and field studies that demonstrate primary degradation half-lives in sediments and soils well in excess of 180 days (e.g. Shaefer & Flaggs (2001a), Feibicke et al., 2009 [ABST], Orihel et al. (2009) [ABST], Muir (2011) [ABST], Sellström et al. (2005), Nyholm et al. (2010b) and Liu et al. (2011)). Sediments and soils are the primary compartments in which the substance resides following release (see Section 3.2). Monitoring data show that decaBDE is widely dispersed in the environment, and that levels in some European estuarine sediments and soils are in the order of a few milligrams per kilogram (parts per million) on a dry weight basis (e.g. Leslie et al., 2008). It is also found at low concentrations in air and is susceptible to long-range atmospheric transport during dry periods, bound to particulates.

Bioaccumulation data are equivocal – there is no reliable fish BCF measurement, but field measurements tend not to indicate any significant biomagnification potential in fish food chains. The situation is different in terrestrial food chains, and monitoring data show that the substance can be taken up by many types of aquatic and terrestrial wildlife species, including in tissues of sensitive life stages such as bird eggs, in many geographical locations. The highest reported concentrations are around 400 µg/kg wet weight basis in some top predators, but they are generally much lower (particularly in the aquatic environment). These concentrations seem to be lower than other substances which are considered very bioaccumulative (ECB, 2007a).

The lack of significant toxicity (as indicated by hazard classification) means that decaBDE does not meet the toxicity (T) criterion of Annex XIII. A number of studies (not considered by the registrants) suggest the possibility of effects in aquatic species (including on the thyroid system) at low concentrations. DecaBDE has also been reported to cause mortalities when injected into chicken (*Gallus gallus*) eggs, raising a concern for avian toxicity. Due to methodological limitations imposed by this poorly soluble substance, repeat studies conducted according to recognised protocols would be needed to confirm the results. However, these new data raise concerns for toxicological effects that had previously been discounted. New data on mammalian toxicity have not been considered in this dossier. The conclusion about the T criterion is therefore only tentative for the time being.

DecaBDE is therefore not considered to meet the PBT or vPvB criteria on the basis of its intrinsic properties. This is consistent with the conclusion drawn by the registrants, although the uncertainty in the T conclusion needs to be considered.

The registrants did not consider the potential for transformation, since registrations were submitted before Annex XIII was revised in 2011.

DecaBDE's very high persistence combined with wide distribution in the environment create a high potential for lifetime exposure and uptake in organisms, and a pool of the substance in many localities that will act as a long-term source of degradation products through both abiotic and biotic transformation.

In the atmosphere, it is likely that decaBDE is partially converted to nonaBDEs (and possibly octaBDEs in much smaller amounts) via photolysis. This is expected to lead to an enrichment of the nonaBDE concentration in atmospheric particulates, which will be deposited to sediments and soils.

The available evidence demonstrates that decaBDE can lose bromine atoms to form nonaBDEs and smaller amounts of octaBDEs under a range of relevant environmental conditions. The studies of Orihel et al. (2009) [ABST], Muir (2011) [ABST] and Huang et al. (2010) in particular suggest that small amounts of hepta- and hexaBDEs will form in aquatic systems and soils under actual or realistic worst case conditions in timescales of a year or less, as follows[61]:

- Although full study results are not yet available, the addition of 2.3 g of decaBDE to a boreal lake (compartment volume 180 m^3) resulted in combined concentrations of hepta- and hexaBDEs in fish of around 5-10 ng/g (µg/kg) dw after three months. This finding is supported by a large number of other studies that demonstrate the potential of fish to metabolically debrominate decaBDE.

- The plant pot experiments conducted by Huang et al. (2010) showed that application of decaBDE to soil at a level of 4.7 mg/kg dw could give rise to an additional 14.5-122 µg/kg dw of tetra- to heptaBDE congeners in the soil over a two-month period when plants are present, depending on the species. The weight percentage formation for each congener group over this period is presented in Table 13. The percentage is calculated by dividing the amount of the congener group at the end of the test with the overall mean decaBDE concentration in the unplanted control treatment (4,830 µg/kg dw).

Table 13: Weight percentage formation of PBDE congeners in soil from the study of Huang et al., 2010

PBDE Group	Radish	Alfalfa	Squash	Pumpkin	Maize	Ryegrass
HeptaBDEs	-	-	-	-	-	-
HexaBDEs	1.8	-	-	1.4	0.4	0.1
PentaBDEs	0.7	0.4	0.2	0.4	0.5	0.5
TetraBDEs	0.1	0.2	<0.1	<0.1	0.2	0.2

[61] Studies using gas chromatography/mass spectrometry (GC/MS) can suffer from injection port or on-column debromination of decaBDE during the analysis (e.g. Lagalante et al., 2011). GC/MS analyses require very high temperatures to vaporize the substance in the injection port and to elute it through the GC column (up to 350°C). Unvapourized decaBDE can adhere to the injection port, causing sample carry-over and serving as a source of degradants. The high elution temperature required and decaBDE's long on-column retention due to its high adsorptive properties can also result in degradation. These problems can be addressed by one (preferably two) toluene purges between samples, frequent cleaning of the injection port, and avoidance of glass wool. Unless these steps are taken, there is a possibility that lower molecular weight PBDE congeners may be formed during the analysis. However, the use of suitable controls can highlight instances where this problem is significant, and the two key studies appear to have taken relevant steps to control analytical artefacts.

Although this experiment may represent worst case conditions, the percentages are likely to be minimum values since other isomers might also have been present that were not quantified, the calculation does not take account of lower congeners found in plant tissues, and transformation could be more extensive over a longer period.

In both cases, the results relate to a single input of decaBDE. A more frequent or constant input of decaBDE might be expected to lead to higher concentrations.

From the analysis in Appendix 1, tetra-, penta-, hexa- and heptaBDEs are considered to meet the Annex XIII criteria for PBT/vPvB substances. Although neither octa- or nonaBDEs are considered to be clear PBT or vPvB substances on the basis of the available data, they are very persistent and it is also likely that they will transform to the lower PBDE congeners of concern under appropriate conditions.

In considering what level of degradation is significant in a regulatory sense, some guidance is provided for registrants by ECHA (2008). This says that if transformation/degradation products with PBT/vPvB properties are being generated in individual amounts ≥ 0.1% (w/w), the parent should generally be treated like a PBT/vPvB substance with regard to emission estimation and exposure control [if the parent is made or imported by an individual registrant in amounts of 10 or more tonnes per year]. No time scale is associated with this percentage. The guidance goes on to say "it may be considered, for the sake of relevance of risk posed by such an amount and the proportionality of assessment effort, to elevate the threshold value above 0.1% … (which) should not exceed 10% (w/w) for the total amount of … all transformation/degradation products with PBT/vPvB properties, and the total amount of (such products) should not exceed 1 tonne/year." The use pattern and potential emissions must be taken into account for this decision, and "careful consideration must be given as to whether the lower 0.1% threshold should apply where uses leading to significant emissions are anticipated" (Section R.11.1, p. 10). This is expanded slightly in Section R.11.2 (p. 55), which says "with regard to the requirements for risk characterisation and nature of risk management measures to be implemented, it may be considered to use a threshold value of 10% (w/w) for the total of all … transformation/degradation products having PBT or vPvB properties, *if it is possible to estimate with sufficient certainty* that ... the total amount of (such products) … do not exceed 1 tonne per year" (italics added). Footnote 13 states that this is not meant to be seen as an 'allowable release' threshold, but rather an administrative tool related to the level of effort needed in the Chemical Safety Assessment relative to other registration requirements.

Given the difficulty in accurately estimating emissions from uncontrolled sources and in quantifying rates of transformation in different compartments (including varying environmental parameters such as redox potential, organic carbon content, etc.), it is not possible to reliably estimate what quantity of transformation products might be formed in a year. It is therefore considered more appropriate to use the lower threshold of 0.1% w/w in this case.

Another consideration is that the threshold is meant to apply to individual transformation products. In this case, each PBDE congener group (tetra-, penta-, hexa- and heptaBDEs) is itself composed of 24 to 46 individual possible isomers. A fixed weight percentage will also represent a different number of moles depending on molecular weight (i.e. 0.1% w/w of an octaBDE congener would represent fewer moles than the same mass of a tetraBDE congener). None of the available experimental studies has attempted to quantify the formation of every possible PBDE congener. In addition, it is not feasible to perform a PBT analysis for each isomer separately, because experimental data are not available on this basis (and it would be disproportionate in terms of cost and time to request studies to allow this to be done). Therefore, the PBDE congener group is used as the unit for application of the individual threshold, since the majority of isomers in each group may be assumed to behave in similar ways. In summary, a threshold for formation of any tetra- to heptaBDE congener group of 0.1% w/w over a timescale of a year or so is appropriate in this case as an indicator for the need to take regulatory action.

On this basis, transformation of decaBDE in soil to PBT/vPvB substances (and precursors) is sufficiently extensive such that it is considered to meet the Annex XIII criteria itself for a PBT/vPvB substance. Transformation in the aquatic compartment and in biota provide additional pathways by which organisms can be exposed to some PBT/vPvB PBDE congeners[62].

It should also be noted that other transformation products detected in studies include polybromodibenzofurans (which can also be formed during plastics processing, e.g. Luijk et al., 1992), hydroxylated-PBDE and methoxy-PBDE derivatives. The PBT profile of such substances is unclear because of a lack of measured data (although some quantitative structure-activity relationship predictions are provided in Appendix 3, and these suggest that some may have PBT profiles of concern). In some cases, some of these substances might be significant products of transformation, but they are rarely considered in degradation experiments, so the amounts that might be formed in the environment are also unknown.

The role of temperature on the transformation process(es) is unclear, although PBDE formation was more extensive over the summer months in the sediment mesocosm studies. The influence of climate change on transformation rates is unknown.

6.1.2 Summary and overall conclusions on the PBT, vPvB properties

DecaBDE is very persistent and widely detected in many environmental compartments (including wildlife species). On the basis of the available data it can be concluded that there is a high probability that decaBDE is transformed in the environment to form substances which themselves have PBT/vPvB properties, or act as precursors to such substances, in individual amounts greater than 0.1% w/w over timescales of a year.

DecaBDE is therefore considered to meet the definition of a PBT/vPvB substance in accordance with Annex XIII of the REACH Regulation, and thereby Article 57(d) and (e)

[62] Environmental monitoring conducted in various countries generally shows either a slowing of the increase in, or a decline in, levels of various lower molecular weight PBDEs in biota, sewage sludge and estuarine environments during the 1990s or early 2000s (e.g. Law et al., 2003 & 2006; Batterman et al., 2007; Gauthier et al., 2008; Ikonomou et al., 2011; Johansson et al. 2011; Olofsson et al., 2012; Crosse et al., 2012; Crimmins et al., 2012). These declines appear to be linked to the declines in use and phasing out of the commercial penta- and octaBDE products around this time. There are no signs that these levels are declining to zero yet as these substances are persistent, and also continue to be released from products still in use (e.g. in articles). Ongoing emissions are likely to mask small levels of transformation, which makes it impossible to draw reliable conclusions from monitoring data.

Appendix 1: PBT profile of PBDEs other than decaBDE

Since tetra- to heptaBDEs are Persistent Organic Pollutants under the Stockholm Convention (UNEP, 2009a & 2009b), it is not the purpose of this appendix to re-open discussions on their properties. However, in a REACH context, the PBT properties of the main identified groups of lower PBDE congeners that may be formed by degradation or metabolism of decaBDE is a critical issue. No PBT assessments were conducted under the Existing Substances Regulation (ESR) because the commercial pentaBDE and octaBDE products were banned before this requirement was introduced. This appendix therefore summarises the available information, although it is not exhaustive.

The primary data supporting this analysis have been previously reviewed in detail in the risk assessment reports produced under the ESR (EC, 2001 & 2003), and by Environment Canada (2006). Further data summaries are provided in two United Nations reports (UNEP, 2006 and 2007) and the Environmental Quality Standard (EQS) fact sheet prepared under the Water Framework Directive (EC, 2011). Some additional data are presented in EFSA (2011). No specific additional literature search has been performed (although where references have been noted as part of the review for the main report, they are briefly mentioned here), and the data summarised in the existing peer reviewed reports have not been re-evaluated. No consideration has been given to triBDE or lower molecular weight PBDEs, since these have generally not been found at significant concentrations in the various studies summarised in the main text.

As only limited amounts of data are available on the properties of individual PBDE congeners, a read-across and interpolation approach has been used to conclude on the properties of the homologue group where appropriate. These substances are structurally similar to one another (they are all brominated diphenyl ethers which vary only in the position of the bromine atoms on the aryl rings) and it would be expected that certain key properties such as water solubility, log K_{ow} and bioaccumulation potential will be similar. It is recognised that individual congeners might have slightly different properties, but in the absence of definitive information, each group of equal molecular weight is assumed to behave in essentially the same way.

The two ESR priority substances were not pure chemicals but contained a mixture of PBDE congeners (see Section 1 of the main text). This needs to be remembered when interpreting data generated using the commercial products.

Substance composition and purity

EC (2001) indicated that the specification of commercial pentaBDE products was generally 50-62 % w/w pentaBDE (CAS No. 32534-81-9) and 24-38 % w/w tetraBDE (CAS No. 40088-47-9). The significant impurities (where stated) comprised some or all of the following:

TriBDE (CAS No. 49690-94-0)	0-1 % w/w
HexaBDE (CAS No. 36483-60-0)	4-12 % w/w
HeptaBDE (CAS No. 68928-80-3)	trace

Example compositions of the commercial octaBDE product are shown in Table A1.1.

Table A1.1: Typical composition of commercial octaBDE products (EC, 2003)

Main components	% by weight			
	up to 1994	1997	2000	2001
PentaBDE	10.5-12.0		1.4-12.0	≤0.5
HexaBDE		5.5		≤12
HeptaBDE	43.7-44.5	42.3	43.0-58.0	≤45
OctaBDE	31.3-35.3	36.1	26.0-35.0	≤33
NonaBDE	9.5-11.3	13.9	8.0-14.0	≤10
DecaBDE	0-0.7	2.1	0-3.0	≤0.7

There are important three-dimensional differences in the structure of individual PBDE congeners due to the ether linkage and location/number of bromine atoms. Molecules can only assume a planar or near planar configuration if there are no bromine atoms in the *ortho*-positions of the aromatic rings. For example, decaBDE is predicted to have a dihedral angle of about 90° and a high barrier to rotation around the ether linkage. The benzene rings of non-*ortho*- substituted PBDEs may assume a near planar configuration with a small dihedral angle. Structural considerations might have some bearing on bioaccumulation potential, due to the influence of molecular cross-sectional diameter on membrane transport, and biotransformation, due to the influence of molecular shape on enzyme binding potential.

Persistence

Tetra- to decaBDEs are predicted by BIOWIN (v.4.00) to be recalcitrant with respect to biodegradation. *In chemico* experiments (Rahm et al., 2005 and Granelli et al., 2012) suggest that nucleophilic substitution and reduction reactivity of PBDEs decreases with loss of bromine atoms. Persistence might therefore be expected to increase in the same way.

No mineralisation of the commercial pentaBDE product was seen over 29 days in an OECD 301B ready biodegradation study (EC, 2001). Degradation of 2,2',4,4'-tetraBDE (BDE-47) – a component of the commercial pentaBDE product – was studied in sediment under anaerobic conditions over a 32-week (224-day) period using the same test protocol as described for decaBDE in the main text (details of this study are reported in EC, 2003). The substance concentration at the end of the test was not statistically significantly different from that at the beginning, and less than 1% mineralisation was seen. HPLC analysis using radiometric detection indicated that some transformation products had been formed by the end of the study, which eluted before the parent compound (up to three such peaks were identified in 26 of the 42 samples analysed, with at least one significant peak in all of the samples from the 500 mg/kg treatment). In summary, this congener meets the Annex XIII criteria for a very persistent (vP) substance.

Only the results of an OECD 301D closed bottle test are available for the commercial octaBDE product (EC, 2003). No mineralisation occurred over a 28-day period indicating that the substance was not readily biodegradable.

Overall, since both a tetraBDE congener and decaBDE (see main text) are considered to be very persistent, it is reasonable to conclude that tetra- to nonaBDE congeners also meet the vP crtieria.

Bioaccumulation

Fish bioconcentration factors

A study using a commercial pentaBDE product was carried out with Common Carp (*Cyprinus carpio*) in a flow-through system over eight weeks (summarised in EC, 2001). The test

substance contained tetra- to hexaBDE congeners, but specific congeners were not identified (only the degree of bromination was given). The major components were a pentaBDE (47.4% w/w) and a tetraBDE (37.7% w/w), with smaller amounts of two other pentaBDEs (7.9 and 1.1% w/w, respectively) and two hexaBDE congeners (2.5 and 2.6% w/w, respectively). Two further components of unknown identity were present at 0.1 and 0.6% w/w, respectively. The method of test solution preparation meant that some of the components may not have been fully dissolved. The reported results were therefore recalculated to take account of the water solubility of the components. These "corrected" BCFs are listed below, and were maximum values:

TetraBDE-1: 66,700 l/kg

PentaBDE-1: 17,700 l/kg

PentaBDE-2: 1,440 l/kg

HexaBDE-1: 5,640 l/kg

HexaBDE-2: 2,580 l/kg

Although equilibrium appears to have been reached for the tetra- and pentaBDE components, fish concentrations measured at the end of the 8-week exposure period were higher than the previous weeks, and so it is possible that they were still increasing, particularly for the hexaBDE congeners. The BCF for the hexaBDE components might not therefore represent a steady state, and could have been higher over a longer timescale.

The values clearly show that the tetraBDE and one of the pentaBDE congeners meet the very bioaccumulative (vB) criterion. The other pentaBDE congener (thought to be BDE-99) was less accumulative, which was consistent with the congener pattern observed in monitoring data for biota (reported in EC, 2001). It can also be concluded that the two hexaBDE congeners meet at least the Annex XIII B criterion, and one of them meets the vB criterion.

A fish bioconcentration study using a commercial octaBDE product and a similar protocol as for pentaBDE is described in EC (2003). The test substance was a mixture of hexa- to nonaBDE congeners. The main components were heptaBDE (47% w/w), two octaBDE congeners (17 and 11% w/w, respectively) and nonaBDE (7% w/w). As for the test with the commercial pentaBDE product, it is likely that some of the components were not fully in solution and so the results were corrected for water solubility. After 8 weeks' exposure, the upper limit of the BCF for the octaBDEs was around 9.5 l/kg. For the heptaBDE component, the upper limit was 36 l/kg. There was some indication of low to moderate accumulation of a hexaBDE component, but a BCF was not estimated. It should be noted that this analysis is dependent on the water solubility of the hepta- and octaBDEs in the test medium, which was assumed to be the same as that in pure water, but is not known. These data suggest that the hepta- and octaBDEs investigated in this study did not meet the B or vB criteria based on fish BCF. However, the study with the commercial pentaBDE product (see above) suggested that the hexaBDE congener concentration had not reached steady state after eight weeks. It is therefore possible that concentrations in this study had not reached steady state either. The water solubility of these congeners is also very low (e.g. EC (2003) cited a measured water solubility of 0.5 µg/l at 25ºC for the commercial octaBDE product), and so aqueous exposure might not be an entirely appropriate route to assess bioaccumulation. An additional consideration is that other studies have shown that carp are capable of metabolising PBDE congeners (e.g. Stapleton et al., 2003 [ABST] and 2004a,b, Zeng et al., 2011), so low tissue concentrations in this species might not be reflective of those in other fish with a lower metabolic capacity.

Given some of the uncertainties in these studies, an analysis has been carried out using the available fish BCF data for tetra-, penta- and hexaBDEs, along with the log K_{ow} values for these substances (originally presented in EA, 2009). The data used in this analysis are summarised in Table A1.2. The BCF values were taken from EC (2002), whilst the log K_{ow} values were taken from a number of sources (given the difficulty in their measurement) as follows:

- Estimates obtained using the USEPA EPIWIN v3.12 program;
- Mean log K_{ow} values estimated using the ALOGPS v2.18 program;
- Values estimated by Ellinger et al. (2003) [ABST] based on total surface area (TSA) correlations with the known log K_{ow} values for tetra- to hexaBDEs; and
- Values determined by Ellinger et al. (2003) [ABST] by a GC/MS method using selected polychlorinated biphenyls with known log K_{ow} values as reference substances.

A plot of measured log BCF against log K_{ow} is shown in Figure A1.1 for each of the data sets.

Table A1.2: Data used to estimate the fish BCF for heptaBDE

Congener	**Predicted log K_{ow}**				**Measured fish BCF (l/kg)**	**Log BCF**
	EPIWIN	**ALOGPS**	**Ellinger et al. (2003) TSA**	**Ellinger et al. (2003) GC/MS**		
TetraBDE	6.77	6.32	6.8	7.4	66,700	4.82
PentaBDE	7.66	7.03	7.3	7.7	17,700	4.24
HexaBDE	8.55	7.60	7.8	8.0	5,640	3.75
HeptaBDE	9.44	8.31	8.3	8.4	-	-

Note: Ellinger et al. (2003) cite ranges of values for each PBDE group as well as specific values for some individual congeners. The latter have been used here as an indication for the group.

These data sets are used in this analysis as they allow log K_{ow} values to be predicted (or measured) for each substance of interest on the same basis. It should be noted, however, that the experimentally derived log K_{ow} is often lower than predicted. For example, measured values of the log K_{ow} of the *commercial* products have been determined as 6.57 for pentaBDE (EC, 2000) and 6.29 for octaBDE (EC, 2003). In addition, EC (2003) gives a log K_{ow} value of 7.14 for a heptaBDE (BDE-183) obtained using a slow-stirring method. (For comparison, values of between 6.27 and 9.97 have been measured for decaBDE – see main text.) Although the agreement between these measured values and the predictions used in the analysis is poor, this does not necessarily adversely affect the BCF values obtained as the log K_{ow} is effectively used only as a scaler in Figure A1.1. It is the relative change in log K_{ow} with increasing bromination that is most important in this approach rather than the absolute log K_{ow} value[63].

The regression line fitted to these data points gives the following relationships:

$$\log BCF = -0.603 \times \log K_{ow} + 8.89 \quad \text{for the EPIWIN values}$$

$$\log BCF = -1.79 \times \log K_{ow} + 18.04 \quad \text{for the Ellinger et al. (2003) GC values}$$

$$\log BCF = -1.07 \times \log K_{ow} + 12.11 \quad \text{for the Ellinger et al. (2003) TSA values}$$

$$\log BCF = -0.837 \times \log K_{ow} + 10.12 \quad \text{for the ALOGPS values}$$

Using these equations, a fish BCF for heptaBDE can be estimated to be around 1,580 l/kg (EPIWIN values), 1,010 l/kg (Ellinger et al. (2003) GC values), 144 l/kg (Ellinger et al. (2003) TSA values) and 1,460 l/kg (ALOGPS values). The median fish BCF for heptaBDEs using this approach is 1,200 l/kg, which does not meet the B criterion. On this basis, the octa- and

[63] Environment Canada (2010) uses slightly lower values for its modelling, based on the Experimental Value Adjustment method in the KOWWIN model, and a log K_{ow} for decaBDE of 8.7. The values are: pentaBDEs 4.3; hexaBDEs 5.1; heptaBDEs 6.0; octaBDEs 6.93.

nonaBDE congeners would have a lower BCF. The validity of this extrapolation approach is unknown.

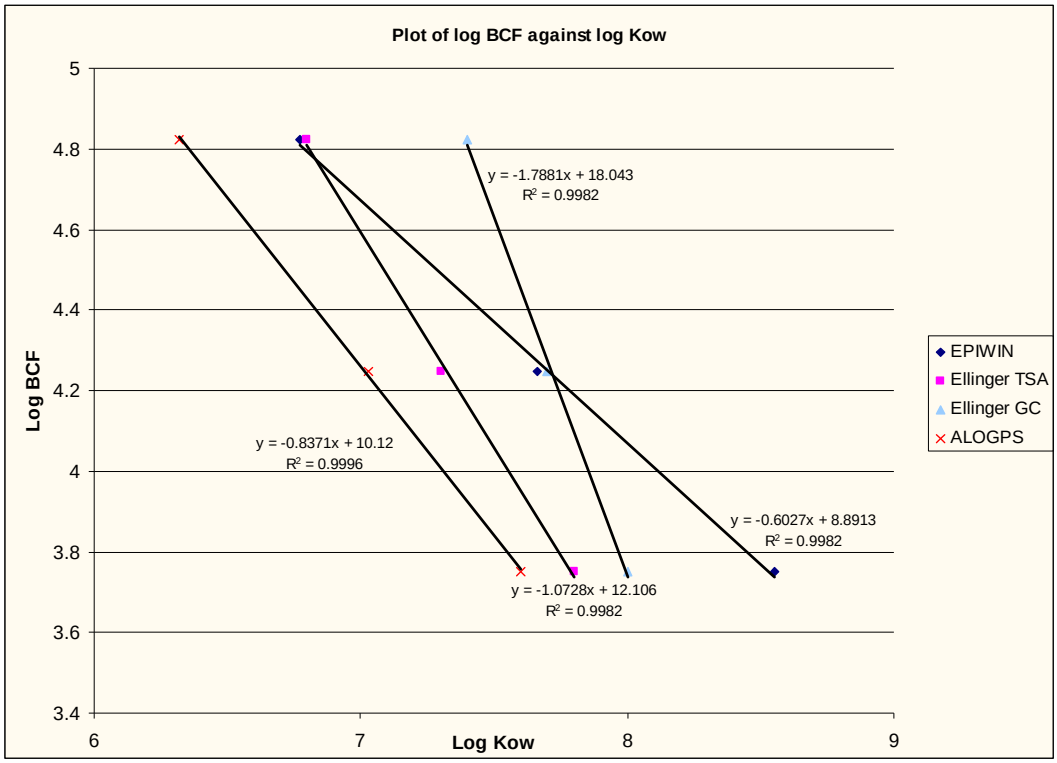


Figure A1.1: Plot of measured log BCF against predicted log K_{ow}

Results from fish feeding studies

No studies have been performed in accordance with the latest draft of the revised OECD 305 Test Guideline that permits exposure via fish food.

Stapleton et al. (2004c) exposed juvenile carp (*Cyprinus carpio*) to a diet spiked with four PBDE congeners (2,4,4'-triBDE [BDE-28], 2,2',4,4'-tetraBDE [BDE-47], 2,2',4,4',5-pentaBDE [BDE-99], and 2,2',4,4',5,5'-hexaBDE [BDE-153]) for 60 days followed by a 40-day depuration period. Concentrations were monitored in whole fish and liver tissues and were found to increase linearly over the duration of the experiment (except for BDE-99). Liver concentrations (on both a wet weight and lipid weight basis) were higher than whole body concentrations, by a factor of 1.1 – 7.7. No phenolic metabolites were detected in blood serum samples (at a detection limit of 1 pg/g ww). Rapid assimilation of BDE-47 was observed relative to the other congeners, whereas apparently no accumulation of BDE-99 occurred over the course of the experiment. The net assimilation efficiencies of BDE-28 and BDE-153 were also low (20 and 4%, respectively). The net assimilation efficiency of BDE-47 increased with time, implying that it was also accumulating as a result of debromination of the higher molecular weight congeners (such as BDE-99; an efficient metabolism of this congener would explain its apparent lack of uptake).

Isohaari et al. (2005) performed a controlled 30-week feeding trial to investigate the uptake of a mixture of PBDEs by Atlantic Salmon (*Salmo salar*). Groups of adult fish with a mean weight of 1.8 kg were exposed to three different concentrations (but similar congener patterns) of

PBDEs in feed[64] under conventional flow-through aquaculture conditions. Three parallel tanks (n = 3) were used per exposure group so that there were nine tanks (65 fish in each). The paper does not mention whether untreated controls were included in the experiment. The mean water temperature was 8.3 °C, oxygen content 9.5 mg/l and flow rate 125 l/min. At the beginning of the trial, two fish from each tank were sampled to form one composite sample of whole fish and one of fillet. After 15 and 30 weeks of exposure, two composite samples (12 whole salmon and 12 filleted salmon) were formed from each tank and analysed for PBDEs. Fish were starved for 3 days before sampling. In addition to the fish samples, fish faeces were collected for analysis after 15 and 30 weeks.

PBDE congeners were detected using gas chromatography with high-resolution mass-spectrometry, operated on a selective-ion recording mode. Quantification was based on the isotope dilution method that compared the peak areas of ^{13}C-labelled internal PBDE standards with the native PBDEs. Fifteen PBDE congeners were quantified this way (1 tri-, 5 tetra-, 4 penta-, 3 hexa- and 2 heptaBDEs). One procedural blank was analysed for every five to ten samples - blank concentrations were far lower than the concentrations in the analysed samples, and so subtracting the blank had no significant effect on the sample concentration.

In exposure group A, the concentration of total PBDEs in whole fish was nearly constant during the trial. However, in exposure groups B and C, the PBDE concentration increased rapidly during the first 15 weeks. During the second 15-week period, the increase rate was slower. Accumulation efficiency (net assimilation, calculated as the ratio of PBDEs retained in whole fish relative to the PBDE intake) was high: 73–133% of the consumed tri- to hexaBDEs accumulated during the 30-week trial. PBDEs excreted into faeces comprised a minor proportion of the mass balance. The unknown residual of the mass balance (i.e. the difference between the PBDE consumption (100%) and the PBDE content in fish tissues and faeces) was 4–7%, suggesting that excretion into water and formation of metabolites other than those that were measured did not play any major role in the fate of PBDEs in this study. The authors suggested that accumulation effieciencies above 100% might be due to *in vivo* formation.

Only one of the two heptaBDEs included in the analysis was detected (BDE-183). Uptake was relatively slow: it was not detected in fish at 15 weeks, but was detectable in the fish in groups B and C by the end of the second period. The reported mean assimilation efficiency of this congener during the second period was 87% (average for Groups A to C, range 80-90%), and the mean fillet to whole fish ratio was 1.30 ± 0.18. The uptake kinetics may have been influenced by changes in the size of the lipid compartment in different tissues during the test[65], as well as the effect of the high food lipid content on bioavailability. The authors considered that the preferential distribution in fillet might indicate that BDE-183 had not yet gained access to the most lipid-rich organs, including liver. It therefore appears unlikely that a steady state had been reached.

Given the apparent lack of control fish, changes in tissue lipid content, lack of data on depuration and uncertainties over steady state, this study cannot be used to estimate a BCF (or biomagnification factor, BMF) for the heptaBDE congener. However, it does show that it can accumulate in fish over a suitable time frame, which appears to be longer than for lower molecular weight PBDE congeners.

Field bioaccumulation factors

[64] The lipid contents of the feed were 33-34%. According to the latest draft of the revised OECD 305 Test Guideline, the test diet should ideally have a total lipid content between 15 and 20%, although this is for much smaller fish.

[65] Although the average lipid contents for whole fish did not change greatly during the trial (~19% fresh weight at the start, 20% by the end), the amount of lipids in fillet doubled between 0 and 15 weeks but then growth of fillet decreased dramatically during the second half of the exposure period (when a large amount of lipids was attributable to the other tissues).

The BCF value measures the uptake into an organism through water (dissolved phase) exposure only. As PBDEs strongly adsorb to suspended matter, it is likely that aquatic organisms will be exposed through both the dissolved phase and through ingestion of particulates, including prey items (e.g. algae, zooplankton, invertebrates, etc.). The accumulation potential for nona-, octa- and heptaBDEs in aquatic organisms may be better expressed in terms of a bioaccumulation factor (BAF) incorporating all possible routes of exposure rather than a BCF, since dietary exposure might be more important for such hydrophobic substances. In addition, food chain accumulation can be measured by estimating biomagnification factors (BMFs) and trophic magnification factors (TMFs) based on measured concentrations and information on feeding relationships or trophic positions (respectively) of species in a food web.

- Wang et al. (2007) studied the bioaccumulation of PBDEs in organisms downstream of a waste water treatment plant (WWTP) in Gaobeidian Lake, Beijing, China. The WWTP treated around one million tonnes of waste water per day (80% from municipal sources) and discharged 30% of the effluent directly into the lake (the remaining effluent was used as cooling water for a nearby power plant before being discharged to the lake, resulting in the temperature of the lake water being above 30°C from May to October). For the study, samples of effluent and lake water were collected in December 2006 and organisms from the aquatic food web were sampled in September 2006. The organisms included spirogyra, March brown[66], coccid[67], zooplankton (*Monia rectirostris*, *Monia micrura* and *Monia macrocopa*), fish (Common Carp (*Cyprinus carpio*), crucian carp (*Carassius auratus*), leather catfish (*Silurus meridionalis*) and java tilapia (*Tilapia nilotica*)) and Chinese soft-shell turtle (*Chinemys reevesii*). In addition a sediment core was collected both upstream and downstream of the WWTP outfall.

  The samples were analysed for tri- to heptaBDEs (and decaBDE). Strict quality controls were used to ensure correct identification and accurate quantification in the analysis and included the analysis of method blanks, etc. The trophic level of each species was determined based on nitrogen isotope ratios. A statistically significant (p <0.05) linear relationship was found between fish BAF (l/kg) and the number of bromine atoms in the molecule (N_{Br}) as follows:

  $$\log BAF = 0.65 - 0.38 \times N_{Br} \qquad (r^2 = 0.439)$$

  Using this equation, the BAF for heptaBDE would be around 6,900 l/kg. Assuming that the relationship holds at higher bromine contents, BAFs of around 2,900 and 1,200 l/kg can be estimated for octaBDE and nonaBDE respectively. It is not clear if these are lipid normalised or whole body wet weight values. It is also unknown whether the concentrations in the water and sediment samples are representative of the exposure situation at the time when biota were sampled three months earlier (when the water temperature and therefore water solubility of the PBDEs might have been higher).

- Gustafsson et al. (1999) exposed blue mussels *Mytilus edulis* to a solution of three PBDEs in a flow-through system without sediment for 44 days, followed by a 26-d depuration period. Estimated BAFs (calculated as the ratio of the uptake clearance rate coefficient to the depuration rate coefficient) were 1.3×10^6 for the tetraBDE, 1.4×10^6 for the pentaBDE and 2.2×10^5 for the hexaBDE. The measurements were based on total water concentrations (i.e. particle associated plus apparently dissolved), but it was not possible

[66] The actual species is unclear: the name "March Brown" appears to relate to the fly *Rithrogena germanica*, but the paper uses the Latin name *Limnodrilus hoffmeisteri* which is an oligochaete worm.

[67] The actual species is unclear: the term Coccid is generally used for various insects of the superfamily *Coccoidea*, including the scale insects and mealybugs.

to assess the relative importance of the ingested food relative to the dissolved fractions as a route of uptake. Based on the same linear extrapolation method using log K_{ow} as for the fish BCF data above, the median mussel BAF for heptaBDE would be around 20,000 l/kg (range 850 – 35,000 l/kg). The median BCF for octaBDE[68] would be 2,400 l/kg (range 130 – 5,400 l/kg). The validity of this extrapolation approach is unknown. However, a study by Riva et al. (2007) suggests that the BCF or BAF for decaBDE in zebra mussels (*Dreissena polymorpha*) may be of the order of 1,000 l/kg (this may be a minimum value due to some uncertaintes that are associated with this study, as summarised in EA, 2009). This is consistent with the extrapolations made here. In addition, it is noted that several studies have reported detection of heptaBDE and higher molecular weight PBDE congeners in molluscs (e.g. Liu et al., 2005).

- La Guardia et al. (2012) investigated PBDE levels in river sediments and mollucs (the filter-feeding bivalve *Corbicula fluminea* and grazing gastropod *Elimia proxima*). Although only a small number of PBDE congeners were analysed in total (two tri-, three tetra-, three penta-, two hexa-, one hepta-, five octa-, three nonaBDEs and decaBDE), the sum of PBDE concentrations at the WWTP outfall was 64,900 ng/g lipid weight in *C. fluminea* and 47,200 ng/g lipid weight in *E. proxima* (decaBDE contributed 48 – 67 % of the amount). The relative abundance in the molluscs was decaBDE >> BDE-209 > BDE-99, BDE-207, BDE-47 > BDE-208 and BDE-100. It is possible that the organisms' guts were contaminated with suspended matter (they were not specifically depurated prior to analysis, but there was a delay of several days between collection and analysis, and they were washed with water in the laboratory). However, when expressed on a dry weight basis, the higher molecular weight congeners show a 1:1 ratio between tissue and sediment concentrations (La Guardia, personal communication). Since the gut only makes up a small proportion of each organism, this implies that a substantial part of the measured amount was present in tissues. Biota-sediment accumulation factors (BSAFs) were greater than one for BDE-47, -99, -100, -153 and -154 (tetra- to hexaBDEs) in both species at almost all locations. A BSAF above one was also obtained for octaBDEs for *C. fluminea* at the outfall site only. BAFs were in the range 550,000 – 6,300,000 for octa- to decaBDE congeners (values read from a graph), calculated based on estimated pore water concentrations, but it is not clear how these related to actual water solubilities.

- DeBruyn et al. (2009) investigated levels of 47 PBDEs in marine mussels (*Modiolus modiolus*) and sediment from fourteen stations near a municipal outfall and three reference locations. Thirty-four PBDE congeners or co-eluting groups of congeners were detected in one or more matrices. The predominant congeners were BDE-47, -99, -100 and decaBDE, accounting for 80-90% of the total PBDEs in all matrices. BSAFs increased with increasing K_{ow} to maximum values of approximately 30-100 for tetra- to hexaBDE congeners, and then declined to a value of approximately 1 for decaBDE. OctaBDEs had BSAFs of about 3 (values read from a graph).

- Wu et al. (2008) investigated a freshwater food web in southern China, including invertebrates (one snail and one prawn species), fish (three carp species) and snakes (two species). A BAF for snails of 199,526 l/kg was estimated for BDE-154 (a hexaBDE).

A number of field studies have investigated the biomagnification potential of PBDEs in various food webs. Fish BMFs above one have been widely reported for tetra- and pentaBDEs (e.g. Kuo et al., 2010a; additional references in EC, 2011). The most commonly detected congeners in aquatic biota are those having three to six bromine substituents, and BDE-47, -100, -154 and -153 have been found to biomagnify in food webs of fish and marine mammals (e.g. Kelly et al., 2008; Muir et al., 2006; Shaw et al., 2009). HeptaBDEs were also reported to biomagnify in fish by Burreau et al., 2004. Nevertheless, the more highly brominated PBDE congeners

[68] The log K_{ow} for octaBDEs is x (EPIWIN) and x (ALOGPS). Ellinger et al. (2003) do not provide data for octaBDEs.

appear to be more frequently observed in terrestrial biota and usually have an elevated contribution to total PBDE burdens in terrestrial animals (e.g. Chen & Hale, 2010).

- Yu et al. (2011) studied a terrestrial food web composed of Common Kestrels *Falco tinnunculus* (n = 23 from a veterinary centre), Eurasian Tree Sparrows *Passer montanus* (n = 40 from nine locations), Brown Rats *Rattus norvegicus* (n = 8, from three locations), grasshoppers (n = several hundred) and dragonflies (n = ~80) from an urban environment in Beijing, China. The kestrels were collected between January 2005 and July 2007; the timing of collection of the other samples is not stated. A field prey delivery study, reinforced by $\delta^{13}C$ and $\delta^{15}N$ analyses, indicated that sparrows were the primary prey items of the kestrels. Total PBDE concentrations were in the following order: kestrel > sparrow > rat > grasshopper and dragonfly. DecaBDE was the dominant congener, and was found together with nonaBDE congeners in all of the samples as well as non-biological matrices (soil and grass). The proportion of decaBDE decreased from more than 60% in soil and grass, to above 50% in the grasshoppers, and approximately 30% in the vertebrates. The authors speculated that this decreasing trend may be partly explained by the debromination of decaBDE at higher trophic levels, since significantly higher nona- to decaBDE ratios found in the bird and rat samples (mean of 1.0, 1.4, and 1.2 for kestrels, sparrows, and rats, respectively) compared to soil and grass samples (mean of 0.40 and 0.53, respectively). The next most abundant congener in kestrels was a hexaBDE (BDE-153). BMFs were calculated as the ratio between the lipid normalized concentrations in the predator and prey. The highest BMF (6.9) was determined for a hexaBDE (BDE-153) in the Sparrow/Common Kestrel food chain. Three octaBDEs (BDE-202, -203, -197), a heptaBDE (BDE-183), a further hexaBDE (BDE-154) and decaBDE itself were also biomagnified in this food chain with BMFs in the range 1.3 to 4.7. Higher BMFs were obtained when prey items from lower trophic levels were considered. In contrast, tetra- and pentaBDEs (BDE-47, -99, and -100) were found to be biodiluted (i.e. BMFs below 1). Measured BMF values for BDE-153, -47, -99 and -100 were consistent with predicted values from a non-steady-state model based on a related kestrel species (Drouillard et al., 2007).

- Voorspoels et al. (2007) estimated BMFs for several tri- to heptaBDE congeners in two predatory bird food chains (passerine/sparrowhawk and rodent/buzzard) and one mammalian food chain (rodent/fox) from Belgium. All congeners (except BDE-28) showed biomagnification in the two avian food chains with the highest BMF obtained for BDE-153 in rodent/buzzard food chain. This supports the findings of Yu et al. (2011).

- Statistically significant TMFs above one were reported for tetra- to hexaBDEs in a Chinese freshwater ecosystem by Wu et al. (2009). Samples included invertebrates (one snail and one prawn species), fish (three carp species) and snakes (two species). Octa- to nonaBDEs were also reported to have TMFs greater than one, but the statistical significance was low. The only heptaBDE included in the analysis (BDE-183) had a TMF below one.

- Zhang et al. (2010) studied a marine food web involving invertebrates (5 species), fish (9 species) and seabirds (2 species). Correlations between lipid normalized PBDE concentrations and trophic levels confirmed that seven out of fourteen PBDE congeners (including hexaBDEs) were biomagnified in the invertebrate-fish-seabird food web, with TMFs above one that were statistically significant in at least one scenario. The single heptaBDE that was monitored (BDE-183) had a TMF above one for the combined food chain, but this was not statistically significant.

- Hu et al. (2010) investigated the trophodynamics of PBDEs in a freshwater food chain involving sixteen aquatic species collected from Baiyangdian Lake, North China. Correlation between lipid-normalized PBDE concentrations and trophic levels determined by stable nitrogen isotope analysis indicated that TMFs were above one for nine PBDE congeners (including two hexaBDEs BDE-153, -154) and a heptaBDE (BDE-183)).

The reliability of some of the reported biomagnification parameters can be affected by methodological limitations inherent in field studies, such as small sample size, collection of unmatched samples over long or unspecified time periods, contamination of blank samples, concentrations near detection limits and extrapolation from single tissue to whole body concentrations. There are no conclusive field BAF data available for individual PBDE congeners. Nevertheless, it can be concluded that tetra- to heptaBDE congeners biomagnify in aquatic and/or terrestrial food chains, and octa- and nonaBDEs appear to biomagnify in terrestrial food chains.

Biota concentration trends

A large amount of environmental monitoring data has demonstrated significant accumulation of tetra- and pentaBDE congeners in a very wide range of wildlife species and tissues (for summaries, see EC, 2001 & 2011 and Environment Canada, 2006). Environment Canada (2006) also indicated that an increasing trend in concentration of hexaBDEs was evident in the blubber of various marine mammals over the previous two decades, and that an analysis of archived herring gull (*Larus argentatus*) eggs (from 1981 to 2000) showed an increasing trend in concentrations of tetra-, penta-, hexa- and heptaBDE congeners.

Mammalian toxicokinetics

EC (2003) considered the mammalian toxicokinetics of the commercial octaBDE product. Only limited data were available. Animal (rat) data showed that absorption occurred following both oral and inhalation exposure, with an accumulation of the "parent compound" [presumably octaBDE congeners] or its metabolites in the liver and also in the adipose tissue and the lung following inhalation administration. The extent of absorption and elimination could not be assessed from the data available. No information on the metabolism of octaBDE was available. Data on human toxicokinetics indicated that the hexa-, hepta-, octa- and nonaBDE components of the commercial product can be absorbed into the body and are distributed to the blood. Distribution to the adipose tissue was evident at least for hexa- and octaBDE. There were no data available on the rate of elimination or accumulation in human adipose tissue. However, it was assumed that in humans octaBDEs might bioaccumulate in these tissues as well. By comparison with lower PBDE congeners, excretion of octaBDEs in breast milk may be anticipated.

EFSA (2011) noted that elimination characteristics of PBDE congeners in animals and humans differ considerably, with elimination half-lives for individual congeners in rats ranging from about 2 to 20 days, whereas for humans maximum values of 926 days (BDE-47, a tetraBDE) to about 4,530 days (BDE-153, a hexaBDE) have been reported. This large difference in kinetics hampers the extrapolation of animal data to humans, and also suggests that wildlife species will also have significant differences in elimination potential.

Modelling predictions

Chemicals that do not accumulate significantly in aquatic organisms might still accumulate in terrestrial organisms. Kelly et al. (2007) proposed that chemicals can be classified into four groups based on their potential to bioaccumulate in air-breathing organisms, using octanol-water and octanol-air partition coefficients (K_{ow} and K_{oa}). Relevant data are summarised in Table A1.3. All of the PBDEs would be categorised as non-polar non-volatiles (log K_{ow} > 5 and log K_{oa} > 6), with a high bioaccumulation potential in both air-breathing organisms and aquatic organisms.

Table A1.3: K_{ow} and K_{oa} data for PBDEs

Congener group	Predicted log K_{ow} [a]	Measured log K_{ow}	Log K_{oa} [b]
TetraBDE	6.32 – 7.4	5.87 – 6.16 (EC, 2003)	10.53 (Harner and Shoeib, 2002)
PentaBDE	7.03 – 7.7	6.46 – 6.97 (EC, 2001)	11.31 (Harner and Shoeib, 2002)
HexaBDE	7.60 – 8.55	6.86 – 7.92 (EC, 2003)	-
HeptaBDE	8.31 – 9.44	7.14 (EC, 2003)	12.78 (Tittlemeier et al., 2002)
OctaBDE	8.9 - 10.33	8.35 – 8.9 (EC, 2003)	13.61 (Tittlemeier et al., 2002)

Note: a – See earlier discussion.
b – These data are cited in Environment Canada (2006), but have not been reviewed for this report.

Environment Canada (2010) performed bioaccumulation modelling predictions for a range of potential decaBDE metabolites including lower molecular weight PBDEs. BAF predictions for penta- to octaBDEs exceeded 5,000 l/kg for the middle trophic level when metabolism rates were taken into account (based on laboratory data), and it was considered that these might even be underestimated. BMF predictions for pentaBDE and higher molecular weight PBDEs in a wolf food chain also exceeded 1.

Other data

Pirard & De Pauw (2007) investigated the absorption, elimination and disposition of six tetra- to heptaBDE in laying chickens (*Gallus domesticus*). Hens were fed a diet containing 3.4 mg/kg feed of PBDEs and 0.95 ng TEQ/kg feed of polychlorodibenzo-*p*-dioxins (PCDDs) and polychlorodibenzofurans (PCDFs). PBDE levels in eggs increased over five weeks and reached 24 µg/g fat. PBDE bioconcentration factors estimated as the ratio between abdominal fat concentration expressed in ng/g fat and feed concentration in ng/g wet weight varied from below 1 for BDE-47 (a tetraBDE) to around 2.2 for hexaBDEs (heptaBDEs had a BCF of 1.0).

Summary

It is clear that tetra- to hexaBDEs have the highest bioaccumulation potential in aquatic food chains, including fish and invertebrates such as molluscs, meeting the B criterion and in some cases the vB criterion.

The estimated fish BCF values for heptaBDE are below 2,000 l/kg. However, this measure possibly underestimates its bioaccumulation potential: the mussel BCF could be in the region of 20,000 l/kg and the fish BAF could be around 7,000 l/kg. In addition, biomagnification has been observed in terrestrial food chains, as well as some fish food chains. Uptake of heptaBDE congeners has been shown to occur in fish, and they can accumulate in mammalian tissues, including breast milk. Modelling predictions also suggest that BAFs and BMFs are above 5,000 l/kg and 1, respectively. Whilst there are uncertainties involved with some of these data, the balance of evidence suggests that the heptaBDE congeners also meet the B criterion.

The categorisation of octaBDEs is less clear. The fish BCF is expected to be significantly below 2,000 l/kg. An extrapolated mussel BCF, based on data for tetra- to hexaBDEs, could be taken to meet the B criterion since it is above 2,000 l/kg. Although there is some uncertainty in this extrapolation, and there are no measured data for octaBDEs, the data are consistent with another study that investigated accumulation of decaBDE in mussels. Field data indicate mollusc BSAFs above one in more than one study, and a high BAF (2,900 l/kg for fish,

>550,000 l/kg for mussels), but the reliability of these estimates is unknown, and there is no guidance about how such values should be judged against the Annex XIII B criterion (in many cases, field BAF data are higher than BCF data, so the implication is that the numerical criteria should be different). There is a lack of evidence for any increasing trends in wildlife tissues, although the high octanol-water and octanol-air partition coefficients suggest a high bioaccumulation potential in air breathing organisms and modelling predictions also suggest that BAFs and BMFs are above 5,000 l/kg and 1, respectively. OctaBDEs have been shown to biomagnify in terrestrial food chains. On balance, the evidence for octaBDEs meeting the B criterion is weaker than for heptaBDEs.

By interpolation with decaBDE, nonaBDEs would not be expected to meet the B criteria on the basis of fish or mollusc BCF, or B(S)AF estimated from field data. However, like decaBDE, there is some evidence that they can be biomagnified in terrestrial food chains.

Overall, the following categorisations can be made for each PBDE congener group based on the available data in comparison with the Annex XIII B/vB criteria (recognising that some specific congeners might not meet the criteria):

TetraBDEs	vB
PentaBDEs	vB
HexaBDEs	B, some are vB
HeptaBDEs	B, based on the balance of evidence
OctaBDEs	probably not B
NonaBDEs	probably not B

Toxicity

Most toxicity data are available for the commercial products rather than individual PBDE congener groups.

Aquatic organisms

Effects data for the commercial pentaBDE product are described in EC (2001). The lowest aquatic NOEC was 5.3 µg/l for *Daphnia magna*. The test substance had the following composition: 33.7% w/w tetraBDE, 54.6% w/w pentaBDE and 11.7% w/w hexaBDE. The commercial substance, including the main tetra- and pentaBDE congener components, therefore meets the T criterion on the basis of aquatic effects. HexaBDE was a significant component of the test substance, so by inference is assumed to meet the T criteria as well in the absence of data to the contrary.

A commercial octaBDE product caused no effects in acute toxicity tests with fish or in longer-term studies with *Daphnia magna*. The test substance was a composite sample from three manufacturers and had the following composition: hexaBDE 5.5% w/w, heptaBDE 42.3% w/w, octaBDE 36.1% w/w, nonaBDE 13.9% w/w, decaBDE 2.1% w/w. As the main components of the test substance were hepta- and octaBDEs it can be tentatively concluded that these components do not meet the T criterion based on the (limited) available information on toxicity to aquatic organisms.

Some studies have also highlighted thyroid disruption as a potential adverse effect in amphibians. For example, Balch et al (2006) reported that a commercial pentaBDE product (DE-71) in the diet at nominal doses of 1,000 and 5,000 µg/g significantly inhibited tail resorption and delayed metamorphosis in *Xenopus laevis* (although it was not confirmed that this was an endocrine-specific effect). Carlsson et al. (2007) reported that dietary exposure to

BDE-99 at 1 mg/g had a similar impact on *X. tropicalis* metamorphosis. As these results were derived without using standard test guidelines, their overall reliability is unclear.[69]

Mammals

The human health classification for the commercial pentaBDE product includes specific target organ toxicity after repeated dose, Category 2 (H373 - May cause damage to organs through prolonged or repeated exposure), as well as Lact. (H362 - May cause harm to breast-fed children). Therefore, the commercial substance, including the main tetra- and pentaBDE congener components, meets the T criterion on the basis of mammalian toxicity data. HexaBDE was a significant component of the test substance, so by inference is assumed to meet the T criteria as well in the absence of data to the contrary.

Annex VI of Regulation (EC) No. 1272/2008 indicates that the commercial octaBDE product is classified as toxic to reproduction Category 1B (H360DF - May damage the unborn child. Suspected of damaging fertility)[70]. This classification means that the commercial octaBDE product meets the Annex XIII T criterion. It is not known which components of the commercial product might have contributed to the toxicity that led to this classification but the two main components to which the animals would have been exposed were the heptaBDEs and octaBDEs[71], and exposure to the hexaBDEs could also have been significant (as these have a higher bioaccumulation potential than the higher molecular weight congeners). It can therefore be concluded that at least hexa-, and probably heptaBDEs meet the T criterion on the basis of mammalian effects (in the absence of information on specific congener groups).

The three commercial PBDE products (penta-, octa- and decaBDE) were all included on the initial EU list of endocrine disrupters in category 2, i.e. *in vitro* data indicated a potential for endocrine disruption in intact organisms (also includes effects *in vivo* that may, or may not, be endocrine-mediated, structural analyses and metabolic considerations) (EC, 2000). Kortenkamp et al. (2012) summarise a large number of more recent studies and reviews that suggest links between PBDE exposure and various endocrine-mediated responses (particularly involving thyroid hormones).

EC (2011) summarises additional mammalian toxicity data that indicate that BDE-99 (2,2',4,4',5-pentaBDE) may also cause neurobehavioural effects at doses in the region of 0.6 mg/kg bw/d in mice following oral exposure (Eriksson et al., 2001b & 2002; Branchi et al., 2002 & 2005). Similar observations have been reported in rats (e.g. Kuriyama et al., 2005; Cheng et al., 2009). Neurotoxic effects in mice have also been reported for hepta-, octa- and nonaBDEs (Viberg et al., 2006). An overview is provided by Costa and Giordano (2007), although a lack of consistency in response has been highlighted by some authors (e.g. Williams and DeSesso, 2010). (Similar studies for decaBDE were also criticised in ECB (2007a).)

EFSA (2011) concluded that the main targets of PBDE toxicity in mammals are the liver, thyroid hormone homeostasis and the reproductive and nervous system, with effects on

[69] As discussed in Section 5 of the main text, recent studies suggest some effects on fish and amphibians exposed to decaBDE at or around the water solubility limit or via the diet over long-term exposures. The difficulty in maintaining test concentrations and non-standard methods mean that these studies would need to be repeated using standardised test guidelines before a decision can be taken about the reliability of the observations. However, they suggest that the T criterion might be met. If this were confirmed, the conclusion would be extrapolated to the other highly brominated PBDE congeners.

[70] The equivalent classification using the criteria in Directive 67/548/EEC is toxic to reproduction Category 2 (R61 – May cause harm to the unborn child) and toxic to reproduction Category 3 (R62 – Possible risk of impaired fertility).

[71] EC (2003) indicates that the composition of the commercial product used in the toxicity test leading to the developmental toxicity classification was 0.2% pentaBDE, 8.6% hexaBDE, 45% heptaBDE, 33.5% octaBDE, 11.2% nonaBDE and 1.4% decaBDE (the composition of the test substance that led to the fertility classification is not reported in EC (2003) but is expected to have been broadly similar).

neurodevelopment as the critical endpoint for the eight PBDE congeners considered (BDE-28 (tri), -47 (tetra), -99 (penta), -100 (penta), -153 (hexa), -154 (hexa), -183 (hepta) and -209). It was also noted that although PBDEs do not induce gene mutations, they cause DNA damage through the induction of reactive oxygen species.

Birds

No avian toxicity data are available from standard test guideline studies. Chen & Hale (2010) provide a review of available data published up to 2009. A number of studies by one laboratory (e.g. Fernie et al., 2009) have demonstrated adverse effects on eggs, nestling growth, laying dates and breeding behaviour in American Kestrels *Falco sparverius* exposed to a commercial pentaBDE product (both via eggs and orally). The lowest-observed-effect level (LOEL) was reported to be 1.8 µg/g egg wet weight or 32 µg/g egg lipid weight. Related studies by the same research group found significant effects on breeding behaviour, clutch size, fertility, circulating testosterone concentrations and male reproductive tract physiology for male birds that had been exposed in the egg (via maternal transfer) at a mean concentration of 1,130 ng/g ww (Marteinson et al., 2010 & 2011). Effects appear to be correlated with hexaBDEs in particular. (The birds were also exposed unintentionally to low concentrations of hexabromocyclododecane, which might have influenced the results.)

An analysis by Johansson et al. (2009) found a negative correlation between the sum of PBDE concentrations in eggs and average productivity for Peregrine Falcon *Falco peregrinus*. The congeners 2,2',4,4',5,5'-hexaBDE, 2,2',4,4',5,6'-hexaBDE and 2,2',3,4,4',5',6-heptaBDE constituted around half of the total PBDE burden in this study. However, this is not proof that the PBDEs were the primary cause of the apparent effect. A similar study appears to have been conducted by Henny et al. (2009) for Osprey *Pandion haliaetus*.

Although no specific criteria exist in Annex XIII for avian toxicity, the REACH Technical Guidance Document (ECHA, 2008) indicates that a chronic NOEC of 30 mg/kg food from an avian sub-chronic, chronic or reproductive toxicity test is equivalent to the T criterion. Assuming that the lowest concentration used by Fernie et al. (2009) of 0.3 mg/kg bw/day represents a NOAEL, and that the conversion factor for chickens (8) is not entirely inappropriate, a NOEC of 2.4 mg/kg food can be estimated. On this basis, the commercial pentaBDE product can be expected to meet the T criterion for birds. There are no toxicity data for individual PBDE congeners, but it appears that hexaBDEs at least may make a significant contribution to the observed toxicity.

As mentioned in the main text, the finding that decaBDE caused mortalities when injected into chicken (*Gallus gallus*) eggs (Silfleet, 2009) raises a concern for avian toxicity for the least bioavailable member of the PBDE group. There are no criteria with which to compare the reported LD_{50} value of 44 µg/egg (740 µg/kg ww), but it might be expected that sub-lethal effects could occur at a lower concentration. It is therefore possible that this finding might trigger the T criterion.

Summary

The following conclusions are reached for each congener group.

TetraBDE T

PentaBDE T

HexaBDE T

HeptaBDE T

OctaBDE Possibly T

NonaBDE The lack of relevant data means that it is not possible to reach a conclusion.

Summary of PBT profiles for specific congener groups

- TetraBDE congeners meet the PBT and vPvB criteria.
- PentaBDE congeners meet the PBT and in some cases the vPvB criteria.
- HexaBDE congeners meet the PBT and in some cases the vPvB criteria.
- HeptaBDE congeners meet the vP and T criteria. They do not appear to meet the B or vB criteria based on an estimated fish BCF, but the balance of available evidence suggests that they can be considered to be B. HeptaBDEs are therefore considered to be a PBT substance.
- OctaBDE congeners meet the vP criteria, but probably do not meet the B criteria. They possibly meet the T criteria[72].
- NonaBDE congeners meet the vP criteria, but probably do not meet the B criteria. There are insufficient data to conclude on T.

The PBT/vPvB nature of the tetra-, penta-, hexa- and heptaBDE congeners has already been recognised by listing them as persistent organic pollutants (POPs) on Annex A of the Stockholm Convention, implemented in the EU as Commission Regulation (EU) No. 757/2010.

Experiments have shown that nonaBDEs can be degraded to octaBDEs by anaerobic bacteria (Gerecke et al., 2005 and 2006). He et al. (2006) and Lee and He (2010) have shown that octaBDE can be biodegraded by anaerobic bacteria collected from a range of locations to hexa-, penta- and tetraBDEs after around six months' incubation at 30°C. Robrock et al. (2008) also elucidated the likely reaction pathway. Stapleton et al. (2004b) showed that Common Carp (*Cyprinus carpio*) exposed to BDE-99 and BDE-183 (a penta- and heptaBDE, respectively) via the diet could metabolise these substances to BDE-47 (a tetraBDE) and BDE-154 (a hexaBDE) respectively. It therefore seems likely that nona- and octaBDEs can also act as precursors to the PBT/vPvB congeners. Indeed, UNEP (2007) concluded that the octa- and nonaBDE congeners are also likely to lead to significant adverse human health and/or environmental effects, such that global action is warranted, due to their transformation to other PBDEs.

[72]The European Commission has proposed to identify octaBDE as a "Priority Hazardous Substance" in the context of the Water Framework Directive, because of its PBT properties (EC, 2012). However, it is understood that this refers to the commercial product, on the basis of its lower molecular weight PBDE content. The Environmental Quality Standard for "brominated diphenyl ether" only concerns the sum of six tetra- to heptaBDE congeners.

Appendix 2: PBDE congener nomenclature

Congener type	No.	Br sites
Mono-BDE	1	2
	2	3
	3	4
Di-BDE	4	2,2′
	5	2,3
	6	2,3′
	7	2,4
	8	2,4′
	9	2,5
	10	2,6
	11	3,3′
	12	3,4
	13	3,4′
	14	3,5
	15	4,4′
Tri-BDE	16	2,2′,3
	17	2,2′,4
	18	2,2′,5
	19	2,2′,6
	20	2,3,3′
	21	2,3,4
	22	2,3,4′
	23	2,3,5
	24	2,3,6
	25	2,3′,4
	26	2,3′,5
	27	2,3′,6
	28	2,4,4′
	29	2,4,5
	30	2,4,6
	31	2,4′,5
	32	2,4′,6
	33	2,3′,4′
	34	2,3′,5′
	35	3,3′,4
	36	3,3′,5
	37	3,4,4′
	38	3,4,5
	39	3,4′,5

Congener type	No.	Br sites
Tetra-BDE	40	2,2′,3,3′
	41	2,2′,3,4
	42	2,2′,3,4′
	43	2,2′,3,5
	44	2,2′,3,5′
	45	2,2′,3,6
	46	2,2′,3,6′
	47	2,2′,4,4′
	48	2,2′,4,5
	49	2,2′,4,5′
	50	2,2′,4,6
	51	2,2′,4,6′
	52	2,2′,5,5′
	53	2,2′,5,6′
	54	2,2′,6,6′
	55	2,3,3′,4
	56	2,3,3′,4′
	57	2,3,3′,5
	58	2,3,3′,5′
	59	2,3,3′,6
	60	2,3,4,4′
	61	2,3,4,5
	62	2,3,4,6
	63	2,3,4′,5
	64	2,3,4′,6
	65	2,3,5,6
	66	2,3′,4,4′
	67	2,3′,4,5
	68	2,3′,4,5′
	69	2,3′,4,6
	70	2,3′,4′,5
	71	2,3′,4′,6
	72	2,3′,5,5′
	73	2,3′,5′,6
	74	2,4,4′,5
	75	2,4,4′,6
	76	2,3′,4′,5′
	77	3,3′,4,4′
	78	3,3′,4,5
	79	3,3′,4,5′
	80	3,3′,5,5′
	81	3,4,4′,5

Congener type	No.	Br sites
Penta-BDE	82	2,2′,3,3′,4
	83	2,2′,3,3′,5
	84	2,2′,3,3′,6
	85	2,2′,3,4,4′
	86	2,2′,3,4,5
	87	2,2′,3,4,5′
	88	2,2′,3,4,6
	89	2,2′,3,4,6′
	90	2,2′,3,4′,5
	91	2,2′,3,4′,6
	92	2,2′,3,5,5′
	93	2,2′,3,5,6
	94	2,2′,3,5,6′
	95	2,2′,3,5′,6
	96	2,2′,3,6,6′
	97	2,2′,3,4′,5′
	98	2,2′,3,4′,6′
	99	2,2′,4,4′,5
	100	2,2′,4,4′,6
	101	2,2′,4,5,5′
	102	2,2′,4,5,6′
	103	2,2′,4,5′,6
	104	2,2′,4,6,6′
	105	2,3,3′,4,4′
	106	2,3,3′,4,5
	107	2,3,3′,4′,5
	108	2,3,3′,4,5′
	109	2,3,3′,4,6
	110	2,3,3′,4′,6
	111	2,3,3′,5,5′
	112	2,3,3′,5,6
	113	2,3,3′,5′,6
	114	2,3,4,4′,5
	115	2,3,4,4′,6
	116	2,3,4,5,6
	117	2,3,4′,5,6
	118	2,3′,4,4′,5
	119	2,3′,4,4′,6
	120	2,3′,4,4′,5
	121	2,3′,4,5′,6
	122	2,3,3′,4′,5′
	123	2,3′,4,4′,5′
	124	2,3′,4′,5,5′
	125	2,3′,4′,5',6
	126	3,3′,4,4′,5
	127	3,3′,4,5,5′

Congener type	No.	Br sites
Hexa-BDE	128	2,2′,3,3′,4,4′
	129	2,2′,3,3′,4,5
	130	2,2′,3,3′,4,5′
	131	2,2′,3,3′,4,6
	132	2,2′,3,3′,4,6′
	133	2,2′,3,3′,5,5′
	134	2,2′,3,3′,5,6
	135	2,2′,3,3′,5,6′
	136	2,2′,3,3′,6,6′
	137	2,2′,3,4,4′,5
	138	2,2′,3,4,4′,5′
	139	2,2′,3,4,4′,6
	140	2,2′,3,4,4′,6′
	141	2,2′,3,4,5,5′
	142	2,2′,3,4,5,6
	143	2,2′,3,4,5,6′
	144	2,2′,3,4,5′,6
	145	2,2′,3,4,6,6′
	146	2,2′,3,4′,5,5′
	147	2,2′,3,4′,5,6
	148	2,2′,3,4′,5,6′
	149	2,2′,3,4′,5′,6′
	150	2,2′,3,4′,6,6′
	151	2,2′,3,5,5′,6
	152	2,2′,3,5,6,6′
	153	2,2′,4,4′,5,5′
	154	2,2′,4,4′,5,6′
	155	2,2′,4,4′,6,6′
	156	2,3,3′,4,4′,5
	157	2,3,3′,4,4′,5′
	158	2,3,3′,4,4′,6
	159	2,3,3′,4,5,5′
	160	2,3,3′,4,5,6
	161	2,3,3′,4,5′,6
	162	2,3,3′,4′,5,5′
	163	2,3,3′,4′,5,6
	164	2,3,3′,4,5′,6
	165	2,3,3′,5,5′,6
	166	2,3,4,4′,5,6
	167	2,3′4,4′,5,5′
	168	2,3′,4,4′,5′,6
	169	3,3′,4,4′,5,5′

Congener type	No.	Br sites
Hepta-BDE	170	2,2′,3,3′,4,4′,5
	171	2,2′,3,3′,4,4′,6
	172	2,2′,3,3′,4,5,5′
	173	2,2′,3,3′,4,5,6
	174	2,2′,3,3′,4,5,6′
	175	2,2′,3,3′,4,5′,6
	176	2,2′,3,3′,4,6,6′
	177	2,2′,3,3′,4,5′,6′
	178	2,2′,3,3′,5,5′,6
	179	2,2′,3,3′,5,6,6′
	180	2,2′,3,4,4′,5,5′
	181	2,2′,3,4,4′,5,6
	182	2,2′,3,4,4′,5,6′
	183	2,2′,3,4,4′,5′,6
	184	2,2′,3,4,4′,6,6′
	185	2,2′,3,3′,5,5′6
	186	2,2′,3,4,5,6,6′
	187	2,2′,3,4′,5,5′,6
	188	2,2′,3,4′,5,6,6′
	189	2,3,3′,4,4′,5,5′
	190	2,3,3′,4,4′,5,6
	191	2,3,3′,4,4′,5′,6
	192	2,3,3′,4,5,5′,6
	193	2,3,3′,4′,5,5′,6
Octa-BDE	194	2,2′,3,3′,4,4′,5,5′
	195	2,2',3,3',4,4',5,6-
	196	2,2′,3,3′,4,4′,5,6′
	197	2,2′,3,3′,4,4′,6,6′
	198	2,2′,3,3′,4,5,5′,6
	199	2,2′,3,3′,4,5,5′,6′
	200	2,2′,3,3′,4,5,6,6′
	201	2,2′,3,3′,4,5′,6,6′
	202	2,2′,3,3′,5,5′,6,6′
	203	2,2′,3,4,4′,5,5′,6
	204	2,2′,3,4,4′,5,6,6′
	205	2,3,3′,4,4′,5,5′,6
Nona-BDE	206	2,2′,3,3′,4,4′,5,5′,6
	207	2,2′,3,3′,4,4′,5,6,6′
	208	2,2′,3,3′,4,5,5′,6,6′
DecaBDE	209	2,2′,3,3′,4,4′,5,5′,6,6′

Appendix 3: PBT profile of some hydroxylated and methoxy-PBDEs

Several studies have shown that decaBDE can be transformed to up to thirteen debrominated phenolic metabolites in mammals (i.e. rats) following oral dosing, including a mono-hydroxy-octaBDE, a mono-hydroxy-nonaBDE, and a mono-hydroxy-mono-methoxy-hexaBDE (e.g. Huwe & Smith, 2007; Mörck et al., 2003; Sandholm et al., 2003). There is some evidence that fish and birds can also form these types of metabolites. Similar lower molecular weight substances occur naturally in some marine species, especially sponges but also acorn worms and green algae (see EC, 2001 and references therein). These are generally mono- or di-hydroxy-diphenyl ethers (and their methylated counterparts) with between four and six bromine atoms per molecule. Their presence in marine sponges appears to be associated with symbiotic cyanobacteria. Many of the compounds have been shown to posses antimicrobial properties (Sharma et al, 1969). It is noted that the analogue substance triclosan (CAS no. 3380-34-5), a mono-hydroxy-trichlorodiphenyl ether, also has antimicrobial activity.

Due to the lack of specific experimental data for these types of compound, a PBT screening exercise of theoretical hydroxylated and methoxylated degradants of decaBDE was undertaken using EPWIN v3.20.

- P screening was based on the BIOWIN v4.10 scoring described in the REACH guidance (ECHA, 2008).
- B screening was based on predicted log K_{ow} value (KOWWIN v1.67), which is consistent with the main B assessment of the debrominated congeners. A bioaccumulation model (BCFBAF v3.01) was also run where the model was valid (predicted log K_{ow} <9). The Arnot & Gobas result without biotransformation was used. N.B. hydroxylated substances might be ionised under environmentally relevant pH conditions, which could affect bioaccumulation potential. This has not been considered in this analysis.
- T was based on predicted acute and chronic toxicity (ECOSAR v0.99h) where this was within the predicted solubility range and also within the log K_{ow} domain of the model. No prediction of potential endocrine disrupting properties was made.

The output of these models is not sensitive to the specific congener SMILES string that is used as the input. The results can be summarised as follows:

Structure	**P?**	**B/vB?**		**T?**
		Based on log K_{ow}	**Based on predicted BCF**	
DecaBDE	P	vB	Not valid	Not T
NonaBDE, mono-hydroxy	P	vB	Not valid	Not T
NonaBDE, mono-methoxy	P	vB	Not valid	Not T
OctaBDE, mono-hydroxy	P	vB	Not valid	Not T
OctaBDE, di-hydroxy	P	vB	Not valid	Not T
OctaBDE, mono-methoxy, mono-hydroxy	P	vB	Not valid	Not T
OctaBDE, mono-methoxy	P	vB	Not valid	Not T
OctaBDE, di-methoxy	P	vB	Not valid	Not T
HeptaBDE, mono-hydroxy	P	vB	Not B	Not T
HeptaBDE, mono-hydroxy, mono-methoxy	P	vB	Not valid	Not T
HeptaBDE, mono-methoxy	P	vB	Not valid	Not T
HeptaBDE, di-hydroxy	P	vB	B (regression) not B (Arnot-Gobas)	Not T
HexaBDE, mono-hydroxy	P	vB	B	Not T

Structure	P?	B/vB?		T?
		Based on log K_{ow}	Based on predicted BCF	
HexaBDE, mono-hydroxy, mono-methoxy	P	vB	B	Not T
HexaBDE, mono-methoxy	P	vB	B (regression) not B (Arnot-Gobas)	Not T
HexaBDE, di-hydroxy	P	vB	vB	T

The results show that all of the structures screen as potentially persistent and all are predicted to have log K_{ow} values above 5.

Bioaccumulation predictions for the di-hydroxy–hexa- and -heptaBDEs suggest they are potentially vB and B, respectively. This is a somewhat simplistic assessment. Environment Canada (2010) took a more sophisticated modelling approach by incorporating potential metabolism. Approximately three quarters of the metabolite BAF predictions exceeded 5,000 l/kg for the middle trophic level when metabolism was included at a rate of 0.02/d. These included hydroxymethoxy-penta- to nonaBDEs, and hydroxy-hexaBDEs. BAFs below 5,000 l/kg were predicted for hydroxy-octa- to nonaBDEs. The conclusion was that a large proportion of metabolites could potentially be bioaccumulative. BMF predictions for a wolf food chain also exceeded 1 for all metabolites.

Only the lower molecular weight substances are predicted to be sufficiently soluble to exhibit aquatic toxicity in the potential T range. Their effects on mammals have not been considered, but some potentially relevant findings are beginning to emerge. For example, several hydroxylated PBDEs have been shown to be potent competitors with thyroxin (T_4) for binding to the plasma transport protein transthyretin and some of them directly bind to thyroid hormone receptors (e.g. Marsh et al., 1998; Meerts et al., 2000 & 2001; Ucán-Marín et al., 2009). Dingemans et al. (2010) also found that hydroxylated metabolites of a tetraBDE (BDE-47) increased the release of calcium ions from intracellular stores at much lower concentrations than the parent congener, which might affect neurotransmitter release. In addition, Usenko et al. (2012) found that hydroxylated tetraBDEs induced developmental arrest in embryonic Zebrafish (*Danio rerio*) in a concentration-dependent manner; the toxicity was greater than for the parent PBDE congener. EC_{50}s were estimated to be in the range 0.96 – 3.8 ppm, with a NOEC of 0.0156 ppm. Genes involved in stress response, thyroid hormone regulation and neurodevelopment were significantly upregulated compared to controls.

In summary, some of these substances have potential PBT/vPvB profiles that are of concern.

SMILES codes

DecaBDE: c1(Br)c(Br)c(Br)c(Br)c(Br)c1Oc2c(Br)c(Br)c(Br)c(Br)c2(Br)

NonaBDE, mono-hydroxy: c1(Br)c(Br)c(Br)c(Br)c(Br)c1Oc2c(Br)c(Br)c(Br)c(Br)c2(O)

NonaBDE, mono-methoxy: c1(Br)c(Br)c(Br)c(Br)c(Br)c1Oc2c(Br)c(Br)c(Br)c(Br)c2(OC)

OctaBDE, mono-hydroxy: c1(Br)c(Br)c(Br)c(Br)c(O)c1Oc2c(Br)c(Br)c(Br)c(Br)c2

OctaBDE, di-hydroxy: c1(Br)c(Br)c(Br)c(Br)c(O)c1Oc2c(Br)c(Br)c(Br)c(Br)c2(O)

OctaBDE, mono-methoxy mono-hydroxy: c1(Br)c(Br)c(Br)c(Br)c(OC)c1Oc2c(Br)c(Br)c(Br)c(Br)c2(O)

OctaBDE, mono-methoxy: c1(Br)c(Br)c(Br)c(Br)c(OC)c1Oc2c(Br)c(Br)c(Br)c(Br)c2

OctaBDE, di-methoxy: c1(Br)c(Br)c(Br)c(Br)c(OC)c1Oc2c(Br)c(Br)c(Br)c(Br)c2(OC)

HeptaBDE, mono-hydroxy: c1(Br)c(Br)c(Br)c(O)cc1Oc2c(Br)c(Br)c(Br)c(Br)c2

HeptaBDE, mono-hydroxy mono-methoxy: c1(Br)c(Br)c(Br)c(OC)cc1Oc2c(Br)c(Br)c(Br)c(Br)c2(O)

HeptaBDE, mono-methoxy: c1(Br)c(Br)c(Br)c(OC)cc1Oc2c(Br)c(Br)c(Br)c(Br)c2

HeptaBDE, di-hydroxy: c1(Br)c(Br)c(Br)c(O)cc1Oc2c(Br)c(Br)c(Br)c(Br)c2(O)

HexaBDE, mono-hydroxy: c1(Br)c(Br)c(Br)cc(O)c1Oc2c(Br)c(Br)c(Br)cc2

HexaBDE, mono-hydroxy, mono-methoxy: c1(Br)c(Br)c(Br)cc(O)c1Oc2c(Br)c(Br)c(Br)cc2(OC)

HexaBDE, mono-methoxy: c1(Br)c(Br)c(Br)ccc1Oc2c(Br)c(Br)c(Br)cc2(OC)

HexaBDE, di-hydroxy: c1(Br)c(Br)c(Br)c(O)cc1Oc2c(Br)c(Br)c(Br)cc2(O)

Log K_{ow} (KOWWIN v1.67)

DecaBDE: log K_{ow} = 12.1 (N.B. This model over-predicts when compared with measured data.)

NonaBDE: log K_{ow} = 11.2

NonaBDE, mono-hydroxy: log K_{ow} = 10.7

NonaBDE, mono-methoxy: log K_{ow} = 11.3

OctaBDE: log K_{ow} = 10.3

OctaBDe, mono-hydroxy: log K_{ow} = 9.8

OctaBDE, di-hydroxy: log K_{ow} = 9.4

OctaBDE, mono-methoxy: log K_{ow} = 10.4

OctaBDE, di-methoxy: log K_{ow} = 10.5

OctaBDE, mono-methoxy mono-hydroxy: log K_{ow} = 9.9

HeptaBDE: log K_{ow} = 9.4

HeptaBDE, mono-hydroxy: log K_{ow} = 9.0

HeptaBDE, mono-hydroxy, mono-methoxy: log K_{ow} = 9.0

HeptaBDE, mono-methoxy: log K_{ow} = 9.5

HeptaBDE, di-hydroxy: log K_{ow} = 8.5

HexaBDE: log K_{ow} = 8.6

HexaBDE, mono-hydroxy: log K_{ow} = 8.1

HexaBDE, mono-hydroxy, mono-methoxy: log K_{ow} = 8.2

HexaBDE, mono-methoxy: log K_{ow} = 8.6

HexaBDE, di-hydroxy: log K_{ow} = 7.6

ECOSAR v0.99h

Results are only presented if the values are below the predicted water solubility. Yellow highlighting shows where the T criterion is met (at a screening level).

DecaBDE: log K_{ow} > applicability domain

NonaBDE, mono-hydroxy: log K_{ow} > applicability domain

NonaBDE, mono-methoxy: Log K_{ow} > applicability domain

OctaBDE, di-hydroxy: log K_{ow} > applicability domain

OctaBDE, mono-hydroxy: log K_{ow} > applicability domain

OctaBDE, mono-methoxy mono-hydroxy: log K_{ow} > applicability domain

OctaBDE, mono-methoxy: log K_{ow} > applicability domain

HeptaBDE, di-hydroxy: log K_{ow} > applicability domain

HeptaBDE, mono-hydroxy: log K_{ow} > applicability domain

HeptaBDE, mono-hydroxy, mono-methoxy: log K_{ow} > applicability domain

HeptaBDE, mono-methoxy: log K_{ow} > applicability domain

HexaBDE, mono-hydroxy: log K_{ow} > applicability domain

HexaBDE, mono-hydroxy, mono-methoxy: log K_{ow} > applicability domain

HexaBDE, mono-methoxy: log K_{ow} > applicability domain

HexaBDE, di-hydroxy

Phenols:	Fish	30-day ChV	0.005
Phenols:	Fish	90-day ChV	0.003
Phenols:	Daphnid	21-day ChV	0.004

Log K_{oa} estimate (KOAWIN v1.10 from KOWWIN and HENRYWIN)

NonaBDE, mono-hydroxy: log K_{oa} = 20.6

NonaBDE, mono-methoxy: log K_{oa} = 18.4

OctaBDE, di-hydroxy: log K_{oa} = 22.8

OctaBDE, mono-hydroxy: log K_{oa} =19.3

OctaBDE, mono-methoxy mono-hydroxy: log K_{oa} = 20.7

OctaBDE, mono-methoxy: log K_{oa} = 17.2

OctaBDE, di-methoxy: log K_{oa} = 18.5

HeptaBDE, mono-hydroxy: log K_{oa} = 18.1

HeptaBDE, mono-hydroxy, mono-methoxy: log K_{oa} = 19.4

HeptaBDE, mono-methoxy: log K_{oa} = 15.9

HeptaBDE, di-hydroxy: log K_{oa} = 21.6

HexaBDE, mono-hydroxy: log K_{oa} = 16.8

HexaBDE, mono-hydroxy, mono-methoxy: log K_{oa} = 18.1

HexaBDE, mono-methoxy: log K_{oa} = 14.6

HexaBDE, di-hydroxy: log K_{oa} = 20.3

BIOWIN v4.10 Results

P screening criteria in REACH guidance R11 (table R. 11.2)

1. Biowin2 (Non-Linear Model Prediction): <0.5
2. Biowin3 (Ultimate Biodegradation Timeframe): <2.2
3. Biowin6 (MITI Non-Linear Model Prediction): <0.5

"P" if BIOWIN 2 + BIOWIN 3 criteria met or if BIOWIN 3 + BIOWIN 6 criteria met

NonaBDE, mono-hydroxy

Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0000)

Biowin3 (Ultimate Biodegradation Timeframe): Recalcitrant (-0.0072)

Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0003)

NonaBDE, mono-methoxy

Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0000)

Biowin3 (Ultimate Biodegradation Timeframe): Recalcitrant (-0.1527)

Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0005)

OctaBDE, mono-hydroxy:

Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0000)

Biowin3 (Ultimate Biodegradation Timeframe): Recalcitrant (0.3031)

Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0008)

OctaBDE, di-hydroxy:

Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0000)

Biowin3 (Ultimate Biodegradation Timeframe): Recalcitrant (0.3242)

Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0007)

OctaBDE, mono-methoxy mono-hydroxy:

Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast (0.00)

Biowin3 (Ultimate Biodegradation Timeframe): Recalcitrant (0.1787)

Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0011)

OctaBDE, mono-methoxy:

Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast (0.00)

Biowin3 (Ultimate Biodegradation Timeframe): Recalcitrant (0.1576)

Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0013)

OctaBDE, di-methoxy:

Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast (0.00)

Biowin3 (Ultimate Biodegradation Timeframe): Recalcitrant (0.0332)

Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0018)

HeptaBDE, mono-hydroxy:

Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0000)

Biowin3 (Ultimate Biodegradation Timeframe): Recalcitrant (0.6135)

Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0020)

HeptaBDE, mono-hydroxy, mono-methoxy

Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0000)

Biowin3 (Ultimate Biodegradation Timeframe): Recalcitrant (0.4890)

Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0028)

HeptaBDE, mono-methoxy

Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0000)

Biowin3 (Ultimate Biodegradation Timeframe): Recalcitrant (0.4680)

Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0031)

HeptaBDE, di-hydroxy

Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0000)

Biowin3 (Ultimate Biodegradation Timeframe): Recalcitrant (0.6345)

Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0018)

HexaBDE, mono-hydroxy

Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0000)

Biowin3 (Ultimate Biodegradation Timeframe): Recalcitrant (0.9238)

Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0048)

HexaBDE, mono-hydroxy, mono-methoxy

Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0000)

Biowin3 (Ultimate Biodegradation Timeframe): Recalcitrant (0.7994)

Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0069)

HexaBDE, mono-methoxy

Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0000)

Biowin3 (Ultimate Biodegradation Timeframe): Recalcitrant (0.7783)

Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0075)

HexaBDE, di-hydroxy

Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0000)

Biowin3 (Ultimate Biodegradation Timeframe): Recalcitrant (0.9449)

Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast (0.0044)

Bioaccumulation

BCF-BAF: EPIWIN (v4.10) indicates that model predictions may be highly uncertain for chemicals that have estimated log K_{ow} values above 9. It is noted that decaBDE is included in the training set for the model, but this is based on it's measured log K_{ow} value not its estimated (log K_{ow} = 12.1). On account of the possible uncertainty, no estimations have been made where the predicted log K_{ow} is above 9 for the substance – this covers all the octa- and nona-brominated substances and some of the hepta-brominated congeners. Instead, the screening criteria of log $K_{ow} \geq 4.5$ and log $K_{ow} \geq 5$ have been used. BCFBAF v3.01 has been run for the hepta- and hexaBDE congeners where log K_{ow} is less than 9.

HexaBDE, mono-hydroxy

Regression-based estimate: BCF = 3981

Upper trophic level, 0 biotransformation rate BCF = 3182

Upper trophic level, including biotransformation BCF = 167

Mid trophic level, including biotransformation BCF = 231

Lower level, including biotransformation BCF = 255

HexaBDE, mono-hydroxy, mono-methoxy

Regression-based estimate: BCF = 3633

Upper trophic level, 0 biotransformation rate BCF = 2866

Upper trophic level, including biotransformation BCF = 103

Mid trophic level, including biotransformation BCF = 141

Lower level, including biotransformation BCF = 156

HexaBDE, mono-methoxy

Regression-based estimate: BCF = 2114

Upper trophic level, 0 biotransformation rate BCF = 1408

Upper trophic level, including biotransformation BCF = 250

Mid trophic level, including biotransformation BCF = 350

Lower level, including biotransformation BCF = 388

HexaBDE, di-hydroxy

Regression-based estimate: BCF = 6843

Upper trophic level, 0 biotransformation rate BCF = 5763

Upper trophic level, including biotransformation BCF = 58

Mid trophic level, including biotransformation BCF = 81

Lower level, including biotransformation BCF = 88

HeptaBDE, mono-hydroxy

Regression-based estimate: BCF = 1458

Upper trophic level, 0 biotransformation rate BCF = 778

Upper trophic level, including biotransformation BCF = 36

Mid trophic level, including biotransformation BCF = 50

Lower level, including biotransformation BCF = 55

HeptaBDE, mono-hydroxy, mono-methoxy

Log K_{ow} >9

HeptaBDE, mono-methoxy

Log K_{ow} >9

HeptaBDE, di-hydroxy

Regression-based estimate: BCF = 2507

Upper trophic level, 0 biotransformation rate BCF = 1796

Upper trophic level, including biotransformation BCF = 14

Mid trophic level, including biotransformation BCF = 19

Lower level, including biotransformation BCF = 21

APPENDIX 5: Abbreviations

°C	Degrees centigrade
Å	Angstrom
a.m.	Ante meridiem
ABS	Acrylonitrile butadiene styrene
[ABST]	Abstract
ACHS	Advisory Committee on Hazardous Substances
AMAP	Arctic Monitoring and Assessment Programme
APP	Ammonium polyphosphate
ASE	Accelerated Solvent Extraction
ATH	Aluminium hydroxide
B	Bioaccumulative
BAF	Bioaccumulation factor
BAPP	Bisphenol A bis(diphenylphosphate)
BCF	Bioconcentration factor
BDDP	Tetrabromobisphenol A bis(2,3-dibromopropyl ether)
BDE	Bromodiphenyl ether
BDF	Bromodibenzofuran
BDP	Bisphenol A bis(diphenylphosphate)
BFR	Brominated flame retardant
BMF	Biomagnification factors
BPADP	Bisphenol A bis(diphenylphosphate)
BSEF	Bromine Science and Environmental Forum
CARACAL	Competent Authorities for REACH and CLP
CDPP	Cresyl diphenylphosphate
CEN	The European Committee for Standardisation
CITI	Chemicals Inspection and Testing Institute (Japan)
CLP	Classification, labelling and packaging (of substances and mixtures)
cm^2	Centimetres squared
cm^3	Cubed centimetres
CoRAP	Community Rolling Action Plan
d	Day

DBBE	Decabromodibenzyl ether (decaBDE)
DBBO	Decabromodibenzyl oxide (decaBDE)
DBDE	Decabromodiphenyl ethane
DBDPE	Decabromodiphenyl ether
DBDPO	Decabromodiphenyl oxide (decaBDE)
DG SANCO	The Health and Consumer Protection Directorate General of the European Commission
DecaBDE	Decabromodiphenyl ether
DEFRA	Department for Environment, Food and Rural Affairs
Des.	Desulfitobacterium
DMPP	N-hydroxymethyl-3-dimethylphosphonopropionamide
DS	Danish standard
dw	Dry weight
ε	molar absorption coefficient
EA	Environment Agency
EBFRIP	European Brominated Flame Retardants Industry Panel
EBP	Decabromodiphenyl ethane
EBTBP	Ethylene bis(tetrabromophthalimide)
EC	European Community
ECB	European Chemicals Bureau
ECHA	European Chemicals Agency
EEE	Electrical and electronic equipment
EFRA	European Flame Retardants Association
EFSA	European Food Safety Authority
EI	Electron ionisation
ELA	Experimental Lakes Area
ELV	End of life vehicles
EN	European Standard
EPA	Environmental Protection Agency
EPDM	Ethylene propylene diene monomer
EQS	Environmental Quality Standard
ESR	Existing Substances Regulations (EEC 793/93)
EBTBP	Ethylene bis(tetrabromophthalimide)

EU	European Union
EVA	Ethylene vinyl acetate
F0	Parental generation
F1	First generation offspring
FLE	Forelimb emergence
g	grammes
GADSL	Global Automotive Declarable Substance List
GC	Gas chromatography
GC/MS	Gas chromatography – mass spectrometry
GC-ECNI/MS	Gas chromatography – electron capture negative ion mass spectrometry
GC-NCI/MS	Gas chromatography – negative chemical ionisation mass spectrometry
GC-μECD	Gas chromatography with microelectron-capture detection
GLP	Good laboratory practice
GPC	Gel permeation chromatography
GPP	Green Public Procurement
ha	Hectare
HBCDD	Hexabromocyclododecane
HDPE	High density polyethylene
HeptaBDE	Heptabromodiphenyl ether
HeptaBDF	Heptabromodibenzofuran
HexaBDE	Hexabromodiphenyl ether
HexaBDF	Hexabromodibenzofuran
HFFR	Halogen free flame retardant
HIPS	High impact polystyrene
HPLC	High performance liquid chromatography
IC	Ion chromaograph
IEC	International Electrochemical Commission
INSTA	Inter Nordic Standard
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
Kg	Kilograms
Km	Kilometres
Koa	Octanol-air partition coefficient

Koc	Organic carbon-water partition coefficient
Kow	Octanol/water partition coefficient (log value)
KPa	Kilopascals
Kp(sed)	Sediment-water partition coefficient
Kp(soil)	Soil-water partition coefficient
Kp(susp)	Suspended sediment-water partition coefficient
l	Litres
LCCP	Long chain chloroparaffins
LOEL	Lowest observed effect level
LOI	Limit of oxygen index
M	Molar
m^2	metres squared (area)
m^3	cubed metres (volume)
MCCP	Medium chain chloroparaffins
Methoxy-PBDEs	Methoxy polybromodiphenyl ethers
MITI	Ministry of International Trade and Industry (Japan)
mg	milligrams
ml	millilitres
Mol	Moles
Mmol	Millimoles
MS	Mass spectrometry
mW	Milli Watts
m/z	mass to charge ratio
n.d.	Not detected
nm	Nanometres
NADPH	Nicotinamide adenine dinucleotide phosphate
NCI	Negative chemical ionization
NOEC	No-observed effect concentration
NonaBDE	Nonabromodiphenyl ether
OCP	Organochlorine pesticides
OctaBDE	Octabromodiphenyl ether
OctaBDF	Octabromodibenzofuran
OECD	Organisation for Economic Co-operation and Development

OEMs	Original equipment manufacturers
p	Statistical probability
P	Persistent
Pa	Pascals
PA	Polyamide
PAH	Polycyclic aromatic hydrocarbon
PBAM	Pentabromobenzylacrylate
PBDD	Polybromodibenzodioxins
PBDF	Polybromodibenzofurans
PBDE	Polybromodiphenyl ether
PBT	Persistent, bioaccumulative and toxic
PC	Polycarbonate
PE	Polyethylene
PEN	Polyethylene naphthalate
PentaBDE	Pentabromodiphenyl ether
PentaBDF	Pentabromodibenzofuran
PET	Polyethylene terephthalate
pg	Picograms
PINFA	Phosphorus, Inorganics and Nitrogen Flame Retardants Association
pKa	Dissociation constant
p.m.	Post meridiem
PMMA	Polymethylmethacrylate
Pmol	Picomole
POP	Persistent organic pollutant
PP	Polypropylene
PPB	Parts per billion
PPE	Polyphenylene ether
PPM	Parts per million
PPO	Polyphenylene oxide
PS	Polystyrene
PVC	Polyvinyl chloride
QSAR	Quantitative structure-activity relationship
r^2	Correlation coefficient

RAR	Risk assessment report
RDP	Resorcinol bis(diphenylphosphate)
REACH	Registration, Evaluation, Authorisation and restriction of Chemicals Regulation (EC 1907/2006)
RoHS	Restriction of Hazardous Substances in Electrical and Electronic Equipment Directive (2002/95/EC and recast as 2011/65/EC)
RPA	Risk and Policy Analysts Limited
rpm	Revolutions per minute
rRNA	Ribosomal ribonucleic acid
rT3	Reverse thyronine
s	Seconds (time)
SAN	Styrene acylonitrile
SBR	Styrene butadiene rubber
SCCP	Short chain chloroparaffins
SME	Small, medium sized enterprise
SVHC	Substances of very high concern
t	Exposure time
T	Toxic (hazard classification)
T4	Thyroxine
TBBPA	Tetrabromobisphenol A
TBBPA-DBPE	Tetrabromobisphenol A bis(2,3-dibromopropyl ether)
TCE	Trichloroethene
TetraBDE	Tetrabromodiphenyl ether
TetraBDF	Tetrabromodibenzofuran
THF	Tetrahydrofuran
THPC-urea products	Tetrakis(hydroxymethyl)phosphonium chloride, oligomeric reaction with urea
TPP	Triphenyl phosphates
TPU	Thermoplastic polyurethanes
TriBDF	Tribromodibenzofuran
TMF	Trophic magnification factors
TTBNP	Tris(tribromoneopentyl) phosphate
TTR	Recombinant transthyretin

UK	United Kingdom
UKFFFSR	UK Furniture and Furnishings (Fire Safety) Regulations (1988)
UPS	Unsaturated polyester resin
UNEP	United Nations Environment Programme
US	United States of America
UV	Ultraviolet
vB	Very bioaccumulative
vP	Very persistent
VCI	German Chemicals Industry Association
VECAP	Voluntary Emissions Control Action Programme
vPvB	Very persistent, very bioaccumulative
W	Watts (power)
W	West (direction)
w/w	Weight per weight
WEEE	Waste electrical & electronic equipment
WWTP	Waste water treatment plant
z	Depth

OTHER INFORMATION

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