ACHS opinion on decabrominated diphenyl ether (decaBDE)

CONTENTS

EXECUTIVE SUMMARY

Hazards associated with decabrominated diphenyl ether (deca-BDE; also referred to as BDE-209) have been studied since the early 1990s. Early risk assessments proved inconclusive; however, new data has been reported since the ACHS was last consulted in 2007 and the committee revisited this topic in March 2010. The question presented to the committee was:

- On the basis of these data, does the ACHS consider deca-BDE to be of an equivalent level of concern to a PBT/vPvB substance for the purposes of the REACH Regulation?

Deca-BDE has the highest molecular weight and level of bromination among polybrominated diphenyl ethers (PBDEs). Poor aqueous solubility makes it difficult to analyse in environmental matrices and its many potential breakdown products, arising from historic and current use of other parent materials as well as from deca-BDE, make it difficult to identify degradation products in the environment. The analytical challenges associated with quantifying congeners differing in degree of debromination mean that most studies have not identified degradation products, or have only analysed a few potential products, and contamination due to previous commercial use of degradation products is also a confounding factor.

The few studies addressing abiotic degradation of deca-BDE indicate that significant abiotic degradation can occur in soil samples containing high concentration of zero-valent iron, iron sulfides or manganese oxides. Photodegradation can also lead to debromination of deca-BDE.

Both anaerobic and aerobic microorganisms can initiate debromination of deca-BDE in the laboratory, aerobic microorganisms significantly faster (days/weeks) than anaerobic microorganisms (months/years). The half life of deca-BDE in aerobic and anaerobic soils with digested or activated sludge has been reported to be >360 days but once again no analysis for degration products was carried out[1]. However, this contrasts with evidence for the formation of substances of concern in both shoots and roots of plants following exposure to deca-BDE. This is a route of direct exposure due to consumption by animals/man.

A large scale Canadian study using mesocosms in semi-natural conditions, which has been reported informally but not yet in peer-reviewed papers, provides substantial evidence of transformation of deca-BDE to breakdown products in surface sediments. The main metabolite detected was nona-BDEs but smaller amounts of octa-BDE were also detected at 1 and 8 months. Tri/tetra and penta-BDEs were also observed but close to the limit of detection.

Monitoring studies provide accumulating (though still indirect) evidence for environmental debromination of deca-BDE, previously only predicted by laboratory experiment. However, the evaluation of temporal changes in debromination products is confounded by changes in use patterns and product purity, and by other commercial PBDE sources (penta-BDE and octa-BDE products are still present in consumer articles sold before their use was banned). There appear to be no data reporting long-term trends in the suggested degradation-specific congeners BDE-126 and 202, although monitoring of these contaminants in sludge and sediments continues.

Metabolites of deca-BDE identified in rats include nona- and octa-BDEs (BDEs 201, 202, 206, 207 and 208) and their methoxy/hydroxylated derivatives, methoxy/hydroxylated hepta-,

[1] It is to be noted that if the half-life were 365 days then 50% would be degraded within 1 year.

hexa- and penta-BDEs, and various polar metabolites. These are consistent with the formation of hydrophilic conjugugates which are subsequently deconjugated by the gut microflora.

The data on metabolic debromination of deca-BDE in birds are limited. Two studies, one in which European starlings were exposed to deca-BDE by means of implant and one examining the homologues patterns of hepta, octa and nona-BDEs in peregrine falcon eggs, provide restricted evidence of possible biotransformation of deca-BDE to at least octa- and nona-BDEs.

Fish can debrominate deca-BDE down to hexa-BDEs but the available data do not identify the final products of metabolism. Lower brominated PBDEs are detected but no hydroxy-BDEs are seen in fish. According to Stapleton (pers. comm.) almost all Cyprinid fish (carp, minnows, zebrafish) can debrominate deca-BDE, as can American eels, sculpins, and rainbow trout.

Invertebrates probably have a very low potential to debrominate deca-BDE. The aquatic toxicity and bioaccumulation potential of PBDEs decreases with increasing bromination and it appears unlikely that deca-BDE will show toxic effects to invertebrates up to its solubility limit.

Deca-BDE is of low toxicity in mammals. Any case for identifying it as an SVHC on the basis of mammalian toxicity would require evidence of metabolic debromination to penta, hexa, hepta or octa-BDEs. Hexa-BDE meets the criteria for a vPvB substance and hepta-BDE can be considered to be equivalent to a PBT/vPvB substance, while both are classified as persistent organic pollutants (POPs) under the Stockholm Convention. However, the status of octa-BDE is less clear. It has not been shown to be mutagenic or carcinogenic but it is a reproductive toxin. Its main effect is developmental although it also has adverse effects on fertility. It should be noted that the interpretation of the toxicity data for commercial octa-BDE is complicated by the fact that the substance is a mixture containing significant amounts of lower PBDEs.

PBDEs may exert neurotoxic effects during the neonatal brain growth spurt in mice, which occurs during the first few weeks of postnatal life in rodents, corresponding to the third trimester of pregnancy and first two years of postnatal life in humans. However, a recent study conducted to Good Laboratory Practice standards did not indicate neurotoxicity following administration of deca-BDE to pregnant female rats and evaluation of the offspring.

Conclusions

The potential for debromination of deca-BDE depends largely upon the medium in/on which it is present and its rate of degradation. The REACH legislation does not specify the level of degradation of a parent molecule to substance(s) meeting the Article 57(a - e) criteria required in order to meet the “substances of equivalent concern” definition. Preparations containing SVHC levels below 0.1% are exempt from REACH authorisation procedures. By analogy, it would appear that a 0.1% transformation rate over a specified time period would require the parent molecule to be regarded as meeting the Article 57(f) requirements.

In the environment, it is expected that deca-BDE will bind to solids in the water column and to particles in the atmosphere. Deca-BDE occurs widely in indoor and outdoor dust and air. Such dust may therefore be considered a significant, uncontrollable and long-term diffuse environmental source of deca-BDE. It is difficult to determine the extent of exposure of such dust to light, but such exposure is likely to occur, especially in the atmosphere. Deca-BDE on indoor dust could therefore be a source of hepta-BDE congeners in the wider environment.

The following questions about deca-BDE and its metabolites remain to be answered:

- What are the rates of formation of penta, hexa, hepta and octa-BDE from deca-BDE during vertebrate metabolism and in the environment?
- What are the rates of formation in sediments of products that meet the SVHC criteria?
- How are the debromination products of deca-BDE distributed in the environment?
- What is the timescale of removal or accumulation of these products in the environment?
- What quantities of these compounds are present in the environment as a consequence of the debromination of deca-BDE (bearing in mind that they may also arise from other sources)?
- What is the biological significance of this process?

Paradoxically, the evidence for environmental degradation provides reassurance that deca-BDE is not extremely persistent, but simultaneously raises concerns about its potential to be transformed to SVHCs. It would appear that deca-BDE lies on the borderline of the "very persistent (vP)" classification. For example, there is evidence for significant breakdown of deca-BDE, yet the resulting half-life in soil is >180 days, meeting the criterion for a vP compound.

The ACHS recognises that this conclusion is, of necessity, based upon the results of a single study. The Huang soil/plant study is a reliable study but was carried out under artificial light and at 20/25°C. It is therefore difficult to extrapolate to rates of formation of the degradation products meeting the SVHC criteria in typical outdoor locations.

Circumstantial evidence indicates that there is potential for deca-BDE to debrominate in the environment to substances that are of concern (e.g. hexa- and heptaBDE). The ACHS recognises that independent verification of the soil/plant study, plus a possible dust study considering photodegradation, may be prudent and desirable. Completion of the Canadian mesocosm study should provide the required quantitative data on sediments. However, the ACHS does not think that additional work should be a reason to delay starting the decision-making process.

Advice to regulators

The ACHS concludes that there is strong, but incomplete, scientific evidence indicating that deca-BDE has the potential to undergo transformation to lower brominated congeners in the environment. The additional data reported since 2007 have added to the concerns expressed previously by the committee. In particular, there is an ever-increasing body of evidence indicating that deca-BDE may be degraded to lower congeners that are SVHCs.

Deca-BDE itself does not meet the current criteria for classification as an SVHC. However, the ACHS is satisfied that there is conclusive qualitative evidence that this compound can undergo degradation to lower brominated congeners. Estimates vary as to the rate and extent to which this degradation is likely to occur in the environment. The committee recognises the difficulty of obtaining quantitative evidence given the physical properties of deca-BDE itself (including low aqueous solubility), the large number of potential breakdown products and the fact that these products can be produced from other parent materials as well as from deca-BDE.

The existence of strong qualitative evidence, together with some quantification in experimental systems, has convinced the ACHS that deca-BDE has the potential to undergo environmental degradation to SVHCs. The remaining question is: To what extent can qualitative evidence be relied upon in the regulatory context? If qualitative evidence is considered sufficient for regulatory purposes, then the ACHS considers that deca-BDE meets the Article 57(f) criteria for classification as a Substance of Equivalent Concern. In this case, the committee recommends timely preparation of a Risk Management Options paper and Annex XV dossier.

INTRODUCTION

Hazards associated with decabrominated diphenyl ether (deca-BDE; also referred to as BDE-209) have been studied since the early 1990s within the context of the former Existing Substances Regulation. However, early risk assessments proved inconclusive. More studies focusing on the properties of deca-BDE (including neurotoxic effects) and its degradation mechanisms followed. The Environment Agency, having reviewed the latest data on persistence, bioaccumulation and toxicity (PBT), has concluded that there is sufficient evidence to support the view that deca-BDE is very persistent (vP) and that it accumulates in wildlife. However, biota levels are not sufficiently high to classify deca-BDE as a very persistent and very bioaccumulative (vPvB) substance under the REACH Regulation (the lack of apparent toxicity means it is not a 'PBT' substance either). Nevertheless, debromination has been reported by many studies and the degradation products generated do have PBT/vPvB properties. The ACHS has therefore been asked whether deca-BDE should be put forward as a 'substance of equivalent concern' as construed by REACH Article 57, namely whether it is a REACH substance of very high concern (SVHC), even if not strictly PBT or vPvB.

The Environment Agency notes that evidence of hazard centers on the potential breakdown products of deca-BDE, particularly penta-, hexa- and hepta-BDE congeners. The latter are classified as persistent organic pollutants under the Stockholm Convention. The extent to which these congeners are formed from deca-BDE and the resulting burden of penta, hexa and hepta-BDEs, which depends on their relative rates of formation and further breakdown, are key points for ACHS in developing its advice.

In the spring of 2007, the ACHS issued advice as follows:

"Overall, the evidence suggests that only small amounts of potentially PBT substances would be formed in the environment. However, there is significant uncertainty regarding all pathways of deca-BDE transformation under environmentally relevant conditions. Thus, if deca-BDE is degradable either in the environment or biota, its lower homologues are likely to be bioaccumulative and toxic. In addition, deca-BDE and particularly its lower homologues are semi-volatile organic compounds and therefore there is a risk that they may undergo long-range transport.

Deca-BDE is of concern because it is highly persistent and is present in a range of biota across large areas of the globe. Emissions to the environment should be controlled on this basis alone. Expensive and time-consuming field studies could establish firm data on which to calculate actual risk factors for both the parent compound and any degradation products which might be PBT. However, none of this extra work would change the conclusion on risk management – the need for reduction in emissions.

The actual degradation rate in the environment remains unknown and can only be resolved by focused measurement of biodegradation products which did not form part of the other commercial mixtures. There is insufficient adequately comprehensive data to settle the PBT or equivalent concern issues. No justification therefore exists to change the current conclusion (i) i.e. there is a need for further information and/or testing." [One member gave a dissenting view and considered that degradation presented an unacceptable risk.]

A considerable amount of new data has been reported since 2007, and the ACHS revisited this topic in March 2010. During these discussions, opinion amongst ACHS members was divided as to whether:

i. The weight of evidence is sufficient to justify the classification of deca-BDE as an SVHC.
ii. Confidence in the data is sufficient to justify preparation of a REACH Annex XV dossier.

The committee felt that a number of serious issues needed to be integrated into the reformulation of its advice. To that end, ACHS work since March 2010 has focused on the formation of PBT/vPvB degradation products from deca-BDE, concentrating on primary data sources and taking into consideration material published up to 31st July 2010. The key question addressed in this document, therefore, is:

- On the basis of these data, does the ACHS consider deca-BDE to be of an equivalent level of concern to a PBT/vPvB substance for the purposes of the REACH Regulation?

The secondary questions which must be answered in order to answer this question are:

i. Can deca-BDE be transformed in the environment to SVHCs?
   The main point at issue in considering whether deca-BDE should be considered as an SVHC is the question of whether it can be debrominated to form hepta, hexa and penta brominated diphenyl ethers (hepta- and hexa-BDEs). On the basis of the available data there is a high probability that deca-BDE is degraded (or metabolised) in the environment to form at least nona-, octa- and hepta-BDE congeners. Hexa- and penta-BDEs may also be formed. Hexa-BDEs meet the vPvB and PBT criteria, and both these and hepta-BDE congeners are listed as persistent organic pollutants (POPs) under the Stockholm Convention. Furthermore, the human health hazard classification for commercial octabromodiphenyl ether (octa-BDE) products satisfies the criteria for identification as an SVHC under REACH Article 57(c), and so the potential biological significance of metabolism to octa-BDE is also considered.
ii. Is this transformation significant? If this cannot be answered, what additional information might be needed for a decision?

In asking these questions the Environment Agency provided the following briefing on the regulatory story to about 2008 (Text Box 1):

Text Box 1: Briefing on regulatory issues associated with deca-BDE

The Environment Agency produced three assessments of the environmental risks of the flame retardant decabromodiphenyl ether (deca-BDE) (CAS no. 1163-19-5) under the Existing Substances Regulation (ESR)[2]. The main concern relates to its potential to form substances that have PBT or vPvB properties, and the ACHS's advice was sought on this issue in Spring 2007.

The Environment Agency commissioned a further review in 2008 to take account of the latest findings. The published report (http://publications.environment-agency.gov.uk/pdf/SCHO0909BQYZ-e-e.pdf)

[2] Available at: http://ecb.jrc.ec.europa.eu/documents/Existing-Chemicals/RISK_ASSESSMENT/REPORT/decabromodiphenyletherreport013.pdf and http://ecb.jrc.ec.europa.eu/documents/Existing-Chemicals/RISK_ASSESSMENT/ADDENDUM/decabromodiphenylether_add_013.pdf

identifies additional data that suggest that the formation of PBT/vPvB substances is environmentally relevant. The ACHS has therefore been asked to provide a new opinion as to whether there is now scientific evidence of probable serious effects to the environment from the use of deca-BDE which give rise to an equivalent level of concern to that of a Substance of Very High Concern (SVHC) as defined in Article 57 of the REACH Regulation (EC) No. 1907/2006.

Persistence

Deca-BDE meets the REACH Annex XIII criteria for a very persistent (vP) substance[3] based on the following:

- The complete lack of degradation in a simulation test with anaerobic freshwater sediment after 32 weeks (224 days) in the dark at 22°C (Schaefer and Flaggs, 2001).
- Very limited degradation over ten months (40 weeks or 280 days) in a sediment microcosm study in the dark at 22°C (Tokarz et al. 2008), giving an estimated half life of 6 – 50 years (average: 14 years). The sediment used in this study had a higher organic carbon content (16.4 per cent) than is usually considered (5 per cent organic carbon content is assumed in the REACH Technical Guidance Document). In other words, adsorption is expected to have been higher and availability lower than under 'typical' conditions.
- Sellström et al. (2005) found that levels in a farm soil were still of the order of milligrams per kilogram dry weight around 20 years after the last known input of contaminated sewage sludge.

Deca-BDE is widely detected in environmental media, including biota and samples from remote regions. It is also apparent that the voluntary point source emission reduction programme instigated by the suppliers has not yet had any clear impact on concentrations in the wider environment away from these point sources. Existing levels in sediments are likely to take many years to dissipate.

Potential for the production of SVHCs by degradation of deca-BDE

Whilst deca-BDE is very persistent, a large number of uncertainties remain about its environmental fate and behavior. Previous ESR risk assessments concluded that the significance of degradation to hazardous polybrominated diphenyl ethers (PBDEs) had not been established. As a result, an environmental monitoring programme is being performed over a ten year time frame to investigate this further. However, since the last ESR report was completed, a significant body of new data has become available and when these are considered alongside the previously reviewed information a number of important conclusions can be reached.

On the basis of the available data there is a high probability that deca-BDE is degraded (or metabolised) in the environment to form at least nona-, octa- and hepta-BDE congeners. There is also new evidence that hexa- and penta-BDEs may also be formed (e.g. the Tokarz et al. (2008) study and the finding of BDE-126 in the industry monitoring programme).

Hexa-BDEs meet the vPvB and PBT criteria, and both these and hepta-BDE congeners[4] are listed as persistent organic pollutants (POPs) under the Stockholm Convention. The human health hazard classification for commercial octabromodiphenyl ether products satisfies the criteria for identification as an SVHC under REACH Article 57c. It is not possible to quantify the amounts of these substances being formed from deca-BDE in the environment with any certainty.

[3] Environmental half-life > 180 days in soil or marine, freshwater or estuarine sediment.

[4] PentaBDE congeners are not discussed in Appendix 1 because there is already wide acceptance that they meet the PBT criteria.

ACTIONS TAKEN BY THE FLAME RETARDANTS INDUSTRY

In the light of evidence about the potential environmental hazards associated with the release of deca-BDE, the flame retardants industry introduced a voluntary emission reduction programme in 2004 (http://www.endsreport.com/15985/decabde-emissions-initiative). This programme, the Voluntary Emissions Control Action Programme (VECAP), aimed to reduce process emissions following concerns over the environmental persistence of deca-BDE and its accumulation in sediments.

The VECAP programme, which is run by the European Brominated Flame Retardants Industry Panel and the Bromine Science and Environmental Forum, began in the UK in 2004 with the aim of extending to France, Germany, Italy, Belgium and the Netherlands by 2007. These six countries account for 95% of EU deca-BDE consumption, which occurs almost exclusively in the textile and plastics sectors. From an initial 80 sites in six European countries, VECAP has grown to cover 135 participating sites (http://www.vecap.info/europe/annual-progress-report/).

Programme members agree to adopt a code of good practice for controlling emissions and measure their current emissions to establish a baseline. Methods include using mass balances and estimates of emissions in effluents based on surrogate measurements such as total bromine content. However, the industry found that analysis of deca-BDE itself was too expensive for general use.

The VECAP programme covers three main brominated flame retardants, deca-BDE, tetrabromobisphenol A and hexabromocyclododecane. The participating sites handle 85% of the total volume of these three brominated flame retardants. The programme sponsors issue annual progress reports (available for downloading from http://www.vecap.info/europe/annual-progress-report/); the 2009 progress report was published in January 2010. The key finding of the report was that "visible reductions in potential emissions to air, land and water were achieved during 2008-2009. The overall potential emissions in Europe for the three main brominated flame retardants, deca-BDE, TBBPA and HBCD, reduced from over 6000 kg surveyed in 2008, to less than 2000 kg in 2009, mainly as result of actions taken at user plants".

Further key findings itemised in the report include:

- Establishment of a year-on-year methodology for comparing potential emissions.
- Awareness that the disposal of industrial chemical packaging is the main potential emission source today, which has been followed by a specific programme targeting such emissions.
- Launch of a VECAP certification scheme with three sites certified so far.

The VECAP website states that "The progress achieved by VECAP participants through the supply chain demonstrates the industry's commitment to promoting safe and environmentally responsible use of its products".

While reductions in deca-BDE emissions in Europe have been achieved by means of voluntary actions, the US is in the process of phasing out the use of deca-BDE altogether. In December 2009, the US EPA made the following announcement (Text Box 2):

Text Box 2: EPA Statement on deca-BDE

On December 17, 2009, as the result of negotiations with EPA, the two U.S. producers of decabromodiphenyl ether (deca-BDE), Albemarle Corporation and Chemtura Corporation, and the largest U.S. importer, ICL Industrial Products, Inc., announced commitments to phase out deca-BDE in the United States.

Deca-BDE is a flame retardant which has been used in electronics, wire and cable insulation, textiles, automobiles and airplanes, and other applications.

The companies have committed to end production, importation, and sales of deca-BDE for most uses in the United States by December 31, 2012, and to end all uses by the end of 2013. The company commitment letters and annual progress reports will be posted to this website.

Steve Owens, EPA Assistant Administrator for the Office of Prevention, Pesticides and Toxic Substances, issued the following statement in response to the announcement: "Though deca-BDE has been used as a flame retardant for years, U.S. Environmental Protection Agency has long been concerned about its impact on human health and the environment. Studies have shown that deca-BDE persists in the environment, potentially causes cancer and may impact brain function. Deca-BDE also can degrade to more toxic chemicals that are frequently found in the environment and are hazardous to wildlife.

"Today's announcement by these companies to phase out deca-BDE is an appropriate and responsible step to protect human health and the environment."

EPA intends to encourage the other minor importers of deca-BDE to join this initiative.

Taken from: http://www.epa.gov/oppt/existingchemicals/pubs/actionplans/deccadbe.html

REGULATORY STATUS AND ISSUES

The ACHS has been asked to consider whether deca-BDE can be considered to be a substance of equivalent level of concern for the purposes of Article 57(f) of the REACH Regulation.

The full text of Article 57(f) is as follows:

"(f) substances — such as those having endocrine disrupting properties or those having persistent, bioaccumulative and toxic properties or very persistent and very bioaccumulative properties, which do not fulfill the criteria of points (d) or (e) — for which there is scientific evidence of probable serious effects to human health or the environment which give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) and which are identified on a case-by-case basis in accordance with the procedure set out in Article 59."

Articles 57 (a) to (e) cover certain intrinsic hazards, as opposed to risk. Deca-BDE does not possess these properties; however, if a substance degrades or is transformed in the environment to degradation products that do have the properties outlined in Articles 57 (a) to (e) then the parent molecule may be considered to be a substance of equivalent concern under Article 57(f). Much further information on this aspect is covered in guidance on authorisation:

- Guidance for the preparation of an Annex XV dossier on the identification of substances of very high concern
  http://guidance.echa.europa.eu/docs/guidance_document/svhc_en.pdf

- Guidance on inclusion of substances in Annex XIV (substances subject to Authorisation
  http://guidance.echa.europa.eu/docs/guidance_document/annex_xiv_en.pdf

On a simple chemical basis deca-BDE may debrominate to a large number of isomers. The key isomers, together with their terminology, are as follows:

Coding Number	Isomers
BDE-209	deca-BDE – parent compound
BDE-206 to BDE-208	3 nonabrominated congeners
BDE-194 to BDE-205	12 octabrominated congeners
BDE-170 to BDE-193	24 heptabrominated congeners
BDE-128 to BDE-169	42 hexabrominated congeners
BDE-82 to BDE-127	46 pentabrominated congeners

A list of all the possible isomer structures is provided in Appendix 1. It should be noted that literature papers also refer to hydroxylated replacements on debromination.

The key information on the above tabulated possible degradation products meeting the legal definitions of Article 57 (a) to (e) may be summarised as follows:

- Hexa-BDEs meet the vPvB and PBT criteria. These and the hepta-BDE congeners are listed as persistent organic pollutants (POPs) under the Stockholm Convention.
- The human health hazard classification for commercial octabromodiphenyl ether products satisfies the criteria for identification as an SVHC under REACH Article 57 (c) (i.e. as a category 2 reprotoxin). The octabromodiphenyl ether product tested was itself a mixture which was considered representative of the commercial products, which in 2001 were specified to be:

	% by weight
§ Decabromo	≤ 0.5
§ Nonabromo	≤ 10
§ Octabromo	≤ 33
§ Heptabromo	≤ 45
§ Hexabromo	≤ 12
§ Pentabromo	≤ 0.5

Hence it is necessary to show that deca-BDE can degrade or be transformed in the environment to at least the octa-, hepta- or to hexabromo isomers in order to trigger potential action under Article 57(f) of REACH. Furthermore, in order to meet the definitions in Article 57(f) such degradation has to occur at more than trivial quantities. Guidance on this interpretation is, to some extent, provided in the REACH Regulation itself. Article 56(6) covers preparations containing SVHC substances and states that they are subject to authorisation unless <0.1% of the SVHC substance is present (unless a lower limit is set by specific concentration limits in the

CLP Regulation). However, REACH does not explicitly address breakdown products that meet the SVHC criteria and while the 0.1% figure can be used as guidance the key question is the time period of their formation in order to meet the Article 57(f) criteria relating to "which give rise to an equivalent level of concern". Clearly if the timescale of formation of 0.1% of degradation products which meet the SVHC criteria is very long then this would not meet the legal test in the wording.

The guidance includes the following noteworthy advice for substances that degrade:

"In addition to the elaborations of PBT and vPvB properties and related considerations described above, there are some other particular situations and circumstances that could lead to a consideration of an equivalent level of concern:

- Substances that are not themselves persistent but have degradation products or metabolites that have PBT or vPvB properties.
- Substances for which it is technically difficult (or impossible) to carry out the necessary testing to confirm whether or not the PBT or vPvB criteria as given in Annex XIII are met but there are indications from other data (e.g. screening data) that they are of equivalent concern.
- Read-across of data for a structurally similar substance with known PBT, vPvB properties, or properties of an equivalent level of concern.

The PBT concept is linked closely to other similar concepts in other international fora, such as the Stockholm Convention on Persistent Organic Pollutants (Stockholm Convention, 2001) and The United Nations Economic Committee for Europe (UNECE) Protocol (UNECE, 1998). Substances already identified as POPs under the Stockholm Convention are not subject to Authorisation under REACH as their production and use are already banned (with only a few exceptions). However the criteria developed under the Convention can be used to identify substances with similar properties, and these could be considered when preparing an Annex XV dossier in combination with some of the other considerations discussed earlier in this section. The criteria used under the Stockholm Convention for identifying potential for long-range environmental transport are summarised in Appendix 5 [of the Guidance]."

Under the REACH restrictions Annex (previously the Market and Use Directive) the following brominated flame-retardants are restricted:

- Diphenylether, pentabromo derivative $C_{12}H_5Br_5O$: banned if present at >0.1% in preparations or articles but recently amended.
- Diphenylether, octabromo derivative $C_{12}H_2Br_8O$: banned if present at >0.1% in preparations or articles but recently amended.

The amendments were, for legal reasons, related to the Restriction of Hazardous Substances (ROHS) Directive. The "whereas" article in the amending REACH Regulation - Commission Regulation (EC) No 552/2009 states:

"In the entries in Annex XVII to Regulation (EC) 1907/2006 for the substances diphenylether, pentabromo derivatives and diphenylether, octabromo derivatives, it should be provided that the restrictions do not apply to articles already in use at the date from which the restriction was

to apply as those substances were incorporated in articles which have a long lifecycle and are sold on the second hand market, such as aeroplanes and vehicles. Moreover, since the use of the substances in electrical and electronic equipments is regulated under Directive 2002/95/EC of the European Parliament and of the Council of 27 January 2003 on the restriction of the use of certain hazardous substances in electrical and electronic equipment (5) that equipment should not be subject to the restrictions concerned."

These congeners can give rise by themselves or following debromination to octa-, hepta-and hexa-brominated isomers. It is therefore difficult to draw conclusions from the literature as to whether the relevant brominated species were formed from deca-BDE, which is a necessary legal requirement to meet the Article 57(f) requirements.

GENERAL COMMENTS

DecaBDE is a polybrominated diphenyl ether (PBDE) and has the highest level of bromination and molecular weight (959) in the group. The water solubility of deca-BDE is very low (below 0.1 µg/l at 25°C) and its Henry's law constant is also low (estimates vary between 0.02 and 44.4 Pa m^3 mol^{-1} at around 21-25°C). Deca-BDE has varying solubility in organic solvents and vehicles, as follows (Table 1):

Table 1: Solubility of deca-BDE in solvents and vehicles

Solvent	Solubility, g/l (temperature not stated)
Anisole	9.4
Tetrahydrofuran (THF)	8.8
Xylene	~8.7
Soya phospholipone:Lutrol (16:34 mixture) in water (concentration 0.11 g/l)	7.0
Dimethylamine (DMA)	6.6
Toluene	4.1
Benzene	~4.8
Methylene bromide	~4.2
Anisole/peanut oil (30:70 mixture)	3.8
Dimethyl sulphoxide (DMSO)	3.5
Dimethyl sulphoxide:peanut oil (50:50 mixture)	2.5
Dimethylamide:polyethylene glycol:water (4:4:1 mixture)	1.9
Peanut oil	<1
Ethyl acetate	<0.8
Acetone	~0.5

The poor solubility of deca-BDE makes it difficult to analyse in environmental materials and biological matrices, and this has led to problems in quantitation with respect to both emissions and degradation. Furthermore, the large number of potential breakdown products and the fact that these products can be produced from other parent materials as well as from deca-BDE contribute to the difficulties experienced in identifying and quantitating degradation products

in the environment. It is difficult to interpret the available studies because most have not looked for degradation products, or only analysed a few of the possible isomers generated. In addition, there are issues of contamination due to previous use of the degradation products giving rise to concern.

Deca-BDE does meet the regulatory criteria for being very persistent and yet the newer studies clearly show progressive degradation to lower brominated substances, which in the case of heptabrominated and hexabrominated degradation products meet the definition of being PBT. Hence it might be a surprise that such a very persistent substance could degrade at a sufficient rate to raise concerns about its PBT degradation products. However even in the Huang plant study where degradation rates appear to range from 6.5% to 32.6% in 60 days the calculation taking an average degradation rate of 20% gives a half-life of 186 days, which is above the legal definition for very persistent of 180 days in soil. In the Huang study only a few of the possible degradation products formed were analysed and so a complete mass balance is not possible but the controls do allow the level of degradation to be estimated.

The recent data discussed in detail below, however, indicate that deca-BDE may undergo measurable environmental transformation. Under normal circumstances this would be considered reassuring, but in the case of deca-BDE it appears likely that the resulting degradation products are SVHCs in their own right. This leads to a paradoxical situation whereby the evidence for environmental degradation provides reassurance that the compound is not persistent, while simultaneously raising concerns about its potential to give rise to SVHCs as a result of transformation. This makes it very difficult to provide a clear answer regarding the environmental hazards associated with deca-BDE despite the numerous investigations which have been carried out. It also causes confusion in that if decaBDE is very persistent whilst some of its breakdown products are only persistent and so one assumes might degrade even quicker then to what level do these lower degradation products actually accumulate in the environment?

EVIDENCE FOR ENVIRONMENTAL DEGRADATION

ABIOTIC DEGRADATION

There is a very limited amount of data on abiotic degradation of deca-BDE. The *State of the Science* report on *Bioaccumulation and Transformation of Decabromodiphenyl Ether* prepared by Environment Canada states that the significance of abiotic degradation is highly uncertain. The lack of conclusive evidence on abiotic processes is due to the limited number of studies published on the subject. However, a careful examination of the existing literature provides some clues on the significance of such processes.

In order to distinguish between biotic and abiotic degradation, the presence of sterilized controls is vital. For example, the primary focus of the work of Tokarz et al.[1] was biotic degradation of deca-BDE. However, examination of a control sample, which was autoclaved several times, showed no significant degradation of deca-BDE. This indicates, in that particular example, that biotic processes are more significant than abiotic processes. Unfortunately, many of the published papers on biotic degradation do not have a proper sterilized control, making it impossible to extract the abiotic data[2].

Two important exceptions to the above point should be mentioned. Firstly, significant abiotic degradation can occur in soil samples containing high concentration of zero-valent iron, iron sulfides or manganese oxides. Secondly, photodegradation can also cause a substantial debromination of deca-BDE. These scenarios are discussed below.

Finally, it is important to recognize the role of soil matrix and composition, which can significantly affect the bioavailability of deca-BDE. In the presence of high concentrations of organic matter and given the scenario of a prolonged residence time, the bioavailability of deca-BDE might be significantly reduced. For example, Klosterhaus and Baker[2] observed that deca-BDE aged in soil has a substantially lower bioavailability than that in freshly spiked samples. Tokarz et al.[1] approximated that debromination kinetics of deca-BDE in sediments are reduced by a factor of 10^6-10^7 because of partitioning and mass-transfer constraints. Many studies use freshly prepared samples where the adsorption of deca-BDE to soil organic matter does not reach equilibrium, so their estimates of bioavailability and degradation are based on a 'worst case scenario', which might not always be the case in realistic environmental conditions. Therefore, one should be careful in using well-controlled laboratory studies to approximate the environmental scenario, given high soil heterogeneity and significant variations in deca-BDE residence time.

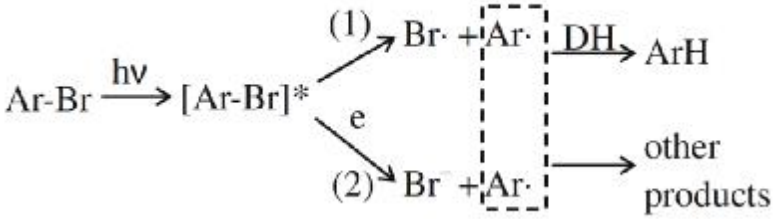
Photodegradation

It is important to distinguish two important scenarios of deca-BDE exposure to sunlight. It appears that when the compound is encapsulated in plastic, no significant degradation occurs. However, when it resides on the surface on the matrix, it can be degraded.

The paper by Kajiwara et al.[3] Suggests that the half life of deca-BDE exposed to light is approximately 51 days, however, that was the case for deca-BDE added to the surface of high impact polystyrene. When considering deca-BDE encapsulated in the matrix, no degradation has been observed. The reason of matrix exhibiting such a profound effect is that it contains other additives, such as coloring agents (pigments), UV absorbers, and stabilizers. These chemicals, according to Kajiwara et al.[3] may have decreased the light penetration depth in each plastic sample, thus decreasing the total light intensity reaching deca-BDE molecules. Even in the worst case scenario, the 51 days half life needs be adjusted upwards, given that the sample has been pulverized and exposed to maximum illumination. Therefore, in more realistic scenarios, the sun exposure (and the degradation rates) of the original, unpulverised plastic will be much lower. The second significant paper in this area (by Stapleton *et al.*) considers the deca-BDE added to the surface of natural dust[4]. The half life was approximately 12.5 days, although these results cannot be directly compared to those of Kajiwara et al.[3], given a difference in underlying substrate and other experimental parameters. The environmental scenario described in this work might be also relevant to deca-BDE degradation in textiles, which is often treated with this chemical. However, a more appropriate strategy would be to conduct additional photochemical studies with deca-BDE treated textile to address this scenario in a more rigorous way. Most of the recently published literature uses experimental conditions which do not adequately approximate the environmental scenarios. For example, Christansson et al.[5] Used deca-BDE dissolved in various solvents. A similar approach has been adopted by various other authors[6-8], complicating interpretation of their results in the context of reasonable environmental conditions. The importance of solvent on the rate of photochemical reaction can be illustrated by the work of Eriksson et al.[9], who compared the photolytic rate of

PBDEs in pure methanol, methanol/ water (8:2) and pure tetrahydrofuran. The authors found that the order of PBDE photolytic rates was tetrahydrofuran > methanol > methanol/water solution, which was in accord with the hydrogen donating ability of the solvents[9].

The lack of data for deca-BDE degradation in appropriate environmental media has important implications for identifying the appropriate reaction pathways and quantifying the mass balance of parent compounds and reaction products. The studies of reaction pathways in various solvents identify the following mechanism[10] the deca-BDE activated by UV light ([Ar–Br]*) can be debrominated either through the C–Br homolysis (step 1) or through the charge transfer from the electron donor (step 2). The aryl radical (Ar•) can then participate in hydrogen abstraction from the hydrogen donor (DH), or undergo other reaction processes including polymerization. The first step of debromination can result in formation of nona-BDE (such as BDE-206, BDE-207 and BDE-208) as described by Stapleton et al.[10,11]. They can further degrade down to hepta-BDE, as indicated by both qualitative measurements and mass balance analysis. Again, the study has been done in solvents other than water, therefore the mass balance and reaction pathways should not be taken as a good approximation of environmental behavior.



It is also important to highlight the effects of the underlying substrate on degradation rate. For example, Soderstrom et al.[12] Found that deca-BDE in natural matrices (sediments and soils) exhibits longer half lives than it does in artificial ones such as silica gel.

In conclusion, it appears that deca-BDE encapsulated in plastic does not undergo detectable photolysis. However, when/if it is released from the matrix, it can undergo significant photodegradation resulting in formation of potentially hazardous by-products. Potential release/degradation of deca-BDE from treated fabric is a scenario which has not been adequately addressed in the literature, despite the fact that this can be a potentially important source of debrominated products. Despite the existence of a number of papers published on this subject, many of them use solvents as a media to study photodegradation and should not be used in approximating the most realistic environmental conditions as they tend to overestimate the degradation rate.

Zero valent iron, manganese oxide and iron sulfide degradation

Abiotic degradation can be significant in soil samples containing high concentration of zero-valent iron or iron sulfides. Keum and Li[13] have studied a system in which approximately 90% of deca-BDE was converted into mono- to hexa-BDEs after 40 days. The analysis of the mass balance for degradation products is shown in the figure below.

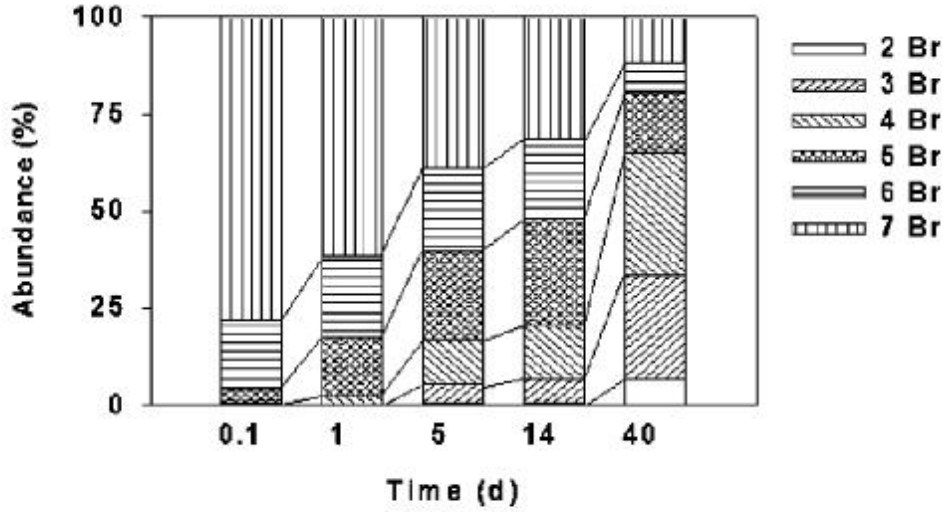


FIGURE 2. Percent distribution of di- to heptabromodiphenyl ethers produced from BDE 209 after treatment with zerovalent iron at 30 °C. All identified di- to heptabromo-BDEs and those of which were tentatively identified by GC-MS were included for the calculation. The total of all these congeners at each given day was calibrated as 100%.

Taken from Keum and Li [13].

It has to be mentioned that the concentration of iron in the samples was excessive and did not correspond to average environmental concentration. The recent paper by Shih and Tai [14] examined zerovalent iron nanoparticles, which is also unrealistic given the enhanced reactivity due to high surface area. However, a control experiment with large iron particles shows that deca-BDE was also degraded on large particles, although at much slower rate. Additional studies involving birnessite (MnO_2)[15] indicated formation of lower brominated products, although no mass balance has been performed. Overall, it does appear that soils containing high concentrations of zero valent iron and iron sulfides can degrade deca-BDE to mono- to hexa-BDE. However, the absence of studies where concentration of iron is close to average concentrations in soil as well as absence of studies where both iron species and soils are combined complicates the data interpretation.

Table 2: Summary of abiotic studies

Congener Group Formed	Zero valent iron [13,15]	Photolysis [3,4]
Nona-BDEs	X	X*
Octa-BDEs	X	X*
Hepta-BDEs	X	X*
Hexa-BDEs	X	X**
Penta-BDEs	X	
Tetra-BDEs	X	

*Stapleton et al.[4]; ** Kajiwara et al.[3]

MICROBES

Several studies have investigated the microbial mediated debromination of deca-BDE under anaerobic conditions. Gerecke et al.[16] Used microflora from sewage sludge to examine the bacterial mediated degradation of deca-BDE (substance purity 97.9%, 2.1 nona-BDE). The results indicated that debromination of deca-BDE did occur, leading to the formation of two nona-BDEs and six octa-BDE congeners, 0.5 nmol of products were generated within 8 months from the debromination of deca-BDE.

The observed first order degradation rate (1.0×10^{-3} day^{-1}) was equivalent to a half life of approximately 690 days. However, this degradation rate was accelerated by the use of primers (one or more of 4-bromobenzoic acid, 2, 6-dibromobiphenyl, tetra-bromobisphenol A, hexa-bromocyclododecane and decabromobiphenyl) to increase degradation potential. Without the use of primers, the observed degradation rate was 50% lower, resulting in a half life of approximately 3.8 years.

In a follow up study, Gerecke et al.[17] Investigated the microbial degradation of deca-BDE (same purity as above) in sewage sludge, again using primers (either 2, 6-debromophenol or 4-bromobenzoic acid) under anaerobic conditions. Half-lives of 700 days (with primer) and 1400 days (without primer) were observed. Monitoring at an operating WWTP found that the concentrations of deca-BDE in sludge decreased between the influent and outlet streams. In the experiments using a primer, deca-BDE transformed slowly to its lower congener BDE-208 (octa-BDE).

In another study it was similarly shown that deca-BDE and octa-BDE could undergo debromination under anaerobic conditions[18]. Deca-BDE (substance purity of 98%) was dissolved in trichloroethylene and inoculated into anaerobic cultures derived from activated sludge. Degradation was only observed with one culture (*S. multivorans*) in which 0.1 mM deca-BDE degraded to non-detectable levels over 2 months. Octa- and hepta-BDEs were detectable at the end of the experiment.

In a study by Tokarz et al.[1] Parallel experiments were conducted using anaerobic sediment microcosms and a co solvent-enhanced biometric system to investigate reductive debromination in deca-BDE. The deca-BDE used in the study contained small amounts of nona-BDE (2.0% BDE-206, 1.9% BDE-207 and 0.9% BDE-208 on a mole fraction basis). Natural sediments with no detectable PBDEs collected from Celery Bog Park, West Lafayette, Indiana were used. PBDEs were then dissolved in a toluene solution added to sediments, then evaporated off. This mixture was then blended with wet sediments. The biomimetic experiment involved the use of Teflon-capped glass vials with 0.03 mM of BDE-209, -99 or -47 mixed with 5.0 mM titanium citrate and 0.2 mM vitamin B12 in 0.33 M TRIZMA buffer solution containing tetrahydrofuran.

This biomimetic system demonstrated reductive debromination at decreasing rates with decreasing bromination (e.g., half life of 18 seconds for deca-BDE and almost 60 days for BDE-47). In natural sediment microcosms, the half life of deca-BDE was estimated to range from 6 to 50 years, with an average of 14 years, based on observations over 3.5 years. At least 12 degradation products were observed but the dominant products were hexa-BDEs after 5 minutes. At longer periods (24 hours) the dominant products present were tetra- (e.g. BDE-47

and BDE-66) and penta-BDEs (e.g. BDE-99 and BDE-119). The proposed pathway for both systems combined was: deca-BDE (BDE-209) > nona-BDEs (BDE-206, -207 -208) > octa-BDEs (BDE-196, -197) > hepta-BDEs (BDE-191, -184, two unknown hepta-BDEs) > hexa-BDEs (BDE-138, -128, -154, -153) > penta-BDEs (BDE-119, -99) > tetra-BDEs (BDE-66, -47, -49) > tri-BDEs (BDE-28, -17). Specifically, at the end of 3.5 years, their analysis of deca-BDE degradation in sediments identified BDE-208, -197, 196, -191, -128, -184, -184, 138, and -128, as well as three unidentified octa-BDEs and two unidentified hepta-BDEs.

These studies show that anaerobic bacteria can initiate debromination of deca-BDE, albeit at a slower rate than photolytic debromination. Given the hydrophobic nature of deca-BDE and the large volumes that enter sewage treatment works, anaerobic degradation may be important in sewage sludge digesters although the residence time of deca-BDE will greatly impact its ability to anaerobically degrade.

It has been presumed that microbial degradation of PBDEs will be low and/or only under anaerobic conditions. It has also been suggested that combinations of anaerobic and aerobic microbial processing may possess the ability to fully degrade PBDEs[19]. To test this, Welsh[20] examined the diversity of sewage microbial communities and their ability to degrade deca-BDE under both anaerobic and aerobic conditions. In this study microorganisms from a sewage bio-solid reactor were isolated, cultured and tested for the capability to degrade BDE-209. Generally, isolates fell into 3 main genera; *Aeromonas spp, Xanthomonas spp* and *Pseudomonas spp*. Of the 16 distinct isolates, six reduced deca-BDE concentrations in the test solutions at both treatments (40 and 80 ppb); while another six did so at one of the treatment levels, but not both. Four of the isolates were not able to degrade deca-BDE at either level under the test conditions. In both treatments some isolates were able to degrade deca-BDE to 50% of initial levels.

It was further demonstrated that debromination occurred in those test tubes where solution levels of deca-BDE decreased. Cultures classified as degraders were tested for evidence of debromination using a silver nitrate (0.01 M) solution. Formation of silver bromine, a yellow precipitate, was used to confirm the presence of free bromine. This verification provided confirmation that deca-BDE had been broken down into lower brominated congeners, although the breakdown products were not identified.

One stark difference between studies of anaerobic degradation of PBDEs and the results by Welsh[20] is the speed at which degradation took place. Anaerobic studies often do not see reduction of parent compounds for months whereas Vonderheide et al.[21] have previously shown that aerobic microbial communities can rapidly degrade parent compounds of BDE-71. While most other studies suggest and even show that degradation rates decrease with higher brominated congeners[1,22], Welsh's study shows that aerobic degradation of deca-BDE by a variety of microorganisms isolated from a common environmental sink, sewage sludge, can occur very quickly, in as little as 20 minutes. The Welsh study appears to be well conducted although it should be noted that this is an examined thesis study and not a peer reviewed paper.

On the basis of the evidence provided so far, it can be concluded that both anaerobic and aerobic microorganisms can initiate debromination of deca-BDE in the laboratory, apparently aerobic

microorganisms faster (i.e. days/weeks) compared to anaerobic microorganisms (months/year).

FUNGI

Evidence of rapid degradation of deca-BDE has been presented by Zhou et al.[23], who evaluated the ability of white rot fungi to degrade deca-BDE in a liquid culture media and the effect of Tween 80 and β-cyclodextrin on the degradation of deca-BDE by white rot fungi. The results showed that test systems with only white rot fungi added showed a decrease of 42.2% over 10 days in the amount of deca-BDE in the test system. The sterile controls showed no significant degradation over time. Tween 80 was found to enhance deca-BDE degradation at an appropriate concentration (maximum degradation 96.5% over 10 days). Cyclodextrin was also shown to enhance deca-BDE degradation (maximum degradation of 78.4% over 10 days). Transformation products were not identified in this study.

The symbiotic relationship between fungi and plant roots, known as arbuscular mycorrhizas, is ubiquitous and may facilitate metabolism of substances such as polycyclic aromatic hydrocarbons in the soil[24]. This effect was considered to be due to the mycorrhiza-associated microflora, since the microbial community structure had an altered phospholipid fatty acid profile. This route of metabolism may be more important than hitherto suspected (see next section).

SOIL AND PLANTS

A recent paper on soil biodegradation kinetics in aerobic and anaerobic soils[25] has confirmed earlier results indicating little degradation of deca-BDE in both aerobic and anaerobic soils with digested or activated sludge. The half life of deca-BDE in this study was >360 days, but degradation products were not analysed and some could still be formed despite this long half life.

However this finding seems to change in a recent key study[26] which included plants in the test system. This was a soil-plant study involving deca-BDE with no contamination from other brominated flame-retardants. Pots were kept in a controlled environment growth chamber for 60d at a light intensity of 250 μmol m^{-2} s^{-1} provided by supplementary illumination with a photoperiod of 14h each study day, at a 25/20°C day/night temperature regime and a relative humidity of 70%. The pots, which contained 600 g spiked soil covered by 65 g nonspiked soil to minimise evaporation and direct photodegration of deca-BDE, were positioned randomly and rerandomised every two days. Distilled water was added as required to maintain moisture content at 60-70% of water holding capacity by regular weighing. Significant deca-BDE degradation was seen in each 60 day study involving radish, alfalfa, squash, pumpkin, maize and ryegrass.

The methodology appears to be sound and this study does allow the determination of the levels of formation of metabolites which are considered to be SVHCs under the REACH legislation. The soil used was a loamy soil without detectable PDBEs. The analytical methodology includes limits of detection for all the species analysed (which included some hydroxylated species) as well as adequate documentation of recoveries. After the deca-BDE was dispersed in the soil at the start of the experiment, analysis detected only very low levels of the nonabrominated isomers and it is unclear whether these were present as impurities in the deca-BDE starting material.

The study did not measure every possible debrominated isomer. It is important to note that the debrominated isomers it did determine were:

- all 3 nona isomers
- 2 of the 12 octa- isomers - considered to be SVHC
- 3 of the 24 hepta-isomers - considered to be SVHC
- 4 of the 42 hexa-isomers - considered to be SVHC
- 5 of the 46 penta-isomers
- 5 of the 42 tetra-isomers
- 2 of the 24 tri-isomers
- 2 of the 12 di-isomers
- 1 of the 3 mono-isomers

Structurally all these relate to the replacement of a bromine atom with a hydrogen atom. The study also determined the presence of 12 hydroxylated species, all of which contain five or less bromine atoms. It did not, however, measure the two species that have been suggested as marker species when examining monitoring data from the environment (BDE-126 and BDE-202).

The additional material from this study[26] provides a full list of substances analysed together with their limits of detection and recoveries from spiked samples. This tabulated data shows high sensitivity compared to measured levels and raises no concerns.

A non-spiked control with plant growth showed minute levels of any of degradation products and only 1.4 ng/g of soil of deca-BDE compared to 4700 ng/g soil in an unplanted but spiked soil control experiment. The initial soil concentration was 5000 ng/g soil and so only some 300 ng/g of deca-BDE seems to be lost to air during the experiments. Hence in the calculations below on the quantities of degradation/transformation products it will be assumed that the starting concentration was 4700 ng/g soil.

The experiments involved the following plants: radish, alfalfa, squash, pumpkin, maize and ryegrass. The plant biomass ranged from 2.8-12.4 g per pot. This is, of course, small compared to the soil weight but as there is clear transfer between the plants and the soil it cannot be determined whether microbes or plants were of most importance in the formation of the degradation/transformation substances. The plants were of course exposed to sunlight and so photodegradation is also possible within the plants.

It is to be noted that the unplanted control showed no significant degradation and what little was seen was to the nona-BDE isomers at the same low level that was present in the spiked soil before the actual growing experiment started. This was therefore also present as background in all the plant experiments. This result agrees with other studies in soil and shows the importance of the presence of plants.

Using Table S3 from the additional study data the following percentages can be calculated for the key substances which meet the SVHC criteria (Table 3). In this calculation a value of 4700 ng/g soil is used to represent no change in the experiment.

Table 3: Concentrations of PBDEs in soil on a dry weight basis.

	BDE Number	Radish	Alfalfa	Squash	Pumpkin	Maize	Ryegrass
Deca-BDE	BDE-209	3073 ng/g	4107.1 ng/g	3402.1 ng/g	4393 ng/g	3612.5 ng/g	3962.6 ng/g
	% BDE degraded	34.6%	12.6%	27.6%	6.5%	23.1%	15.7%
Nona-BDE isomers	BDE-206	111.1 ng/g	111.3 ng/g	102.3 ng/g	83.7 ng/g	125.1 ng/g	147.2 ng/g
	BDE-207	71.6 ng/g	62 ng/g	63.7 ng/g	55.2 ng/g	62.8 ng/g	80.6 ng/g
	BDE-208	52 ng/g	42.6 ng/g	36.2 ng/g	34.5 ng/g	43.4 ng/g	49.1 ng/g
	Total nona isomers	234.7 ng/g	215.9 ng/g	202.2 ng/g	173.4 ng/g	231.3 ng/g	276.9 ng/g
	% degraded to nona	5.0%	4.6%	4.3%	3.7%	4.9%	5.9% ng/g
Octa-BDE isomers	BDE-196	1.5 ng/g	83.2 ng/g	77.0 ng/g	84.4 ng/g	82.2 ng/g	74.1 ng/g
	BDE-197	74.8 ng/g	69.0 ng/g	66.1 ng/g	71.2 ng/g	69.3 ng/g	68.2 ng/g
	Total octa isomers	76.3 ng/g	152.2 ng/g	143.1 ng/g	155.6 ng/g	151.5 ng/g	142.3 ng/g
	% degraded to octa	1.6%	3.2%	3.0%	3.3%	3.2%	3.0%
Hepta-BDE isomers	There are no figures for the hepta isomers in soil						
Hexa-BDE isomers	BDE-138	61.3 ng/g	nd	nd	nd	nd	4.5 ng/g
	BDE-153	0.8 ng/g	nd	nd	43.6 ng/g	nd	nd
	BDE-154	23.2 ng/g	nd	nd	23.8 ng/g	19.3 ng/g	1.9 ng/g
	Total hexa isomers	85.3 ng/g	nd	nd	67.4 ng/g	19.3 ng/g	6.4 ng/g
	% degraded to hexa	1.8%	nd	nd	1.4%	0.4%	0.1%

(adapted from Table S3 of Huang et al.[26])

It can clearly be seen that considerable transformation to debrominated species has occurred in the soil, ranging from 6.5% to 34.6% with the average for the six plants being 20%. This transformation has occurred in only 60 days and does not involve photodegration in the soil. The degradation to substances of concern in a period of 60 days is also considerable:

- For the octa-BDE isomers this ranges from 1.6 to 3.2%. It should be recalled that only 2 of the potential 12 octa isomers are subject to analysis.
- No hepta-BDE isomers were found in the soil but levels of the hexa-BDE isomers ranged from 0.1% to 1.8%. Again only 4 of the 42 isomers were subject to analysis.

Analysis in the roots and shoots of plants is also informative (Tables 4 and 5).

Table 4: Concentrations of PBDEs in shoots on a dry weight basis.

	BDE Number	Radish	Alfalfa	Squash	Pumpkin	Maize	Ryegrass
Deca-BDE	BDE-209	320.4 ng/g	490.1 ng/g	225.7	245.8	268.9	177.9
Nona-BDE isomers	BDE-206	168.4 ng/g	39.7 ng/g	141.5	46.1	119.3	78.8
	BDE-207	177.2 ng/g	50.4 ng/g	172.2 ng/g	51.6 ng/g	69.4 ng/g	100.4 ng/g
	BDE-208	11.4 ng/g	3.1 ng/g	3.4 ng/g	3.1 ng/g	2.9 ng/g	2.6 ng/g
	Total nona isomers	357.0 ng/g	93.2 ng/g	317.1 ng/g	100.8 ng/g	191.6 ng/g	181.8 ng/g
	Ratio to BDE-209	1.1	0.2	1.4	0.4	0.7	1.0
Octa-BDE isomers	BDE-196	86.2 ng/g	19.3 ng/g	69.8 ng/g	23.7 ng/g	29.6 ng/g	35.2 ng/g
	BDE-197	64.0 ng/g	nd	nd	nd	nd18.2	nd
	Total octa isomers	150.2 ng/g	19.3 ng/g	69.8 ng/g	23.7 ng/g	29.6 ng/g	35.2 ng/g
	Ratio to BDE-209	0.5	0.0	0.3	0.1	0.1	0.2
Hepta-BDE isomers	BDE-191	62.3 ng/g	nd	59.6 ng/g	21.7 ng/g	nd	nd
	BDE-183	17.4 ng/g	nd	nd	nd	15.2 ng/g	28.3 ng/g
	Total hepta isomers	79.7 ng/g	0.0 ng/g	59.6 ng/g	21.7 ng/g	15.2 ng/g	28.3 ng/g
	Ratio to BDE-209	0.2	0.0	0.3	0.1	0.1	0.2
Hexa-BDE isomers	BDE-138	nd	nd	16.3 ng/g	nd	nd	26 ng/g
	BDE-156	nd	nd	nd	11.8 ng/g	nd	22.2 ng/g
	BDE-153	61 ng/g	nd	nd	11 ng/g	10.9 ng/g	20.7 ng/g
	BDE-154	nd	nd	33.3	5.8	6	11.1
	Total hexa isomers	61 ng/g	0.0 ng/g	49.6 ng/g	28.6 ng/g	16.9 ng/g	80 ng/g
	Ratio to BDE-209	0.2	0.0	0.2	0.1	0.1	0.4
Hexa- plus Hepta-BDE isomers	Total Hexa + Hepta	140.7 ng/g	0.0 ng/g	109.2 ng/g	50.3 ng/g	32.1 ng/g	108.3 ng/g
	Ratio to BDE-209	0.4	0.0	0.5	0.2	0.1	0.6

(adapted from Table S4 of Huang et al.[26])

Table 5: Concentrations of PBDEs in roots on a dry weight basis.

	BDE Number	Radish	Alfalfa	Squash	Pumpkin	Maize	Ryegrass
Deca-BDE	BDE-209	513.2 ng/g	566.5 ng/g	1946.3 ng/g	2088.1 ng/g	1187.6 ng/g	1878.2 ng/g
Nona-BDE isomers	BDE-206	255.0 ng/g	119.1 ng/g	353.2 ng/g	362.1 ng/g	107.2 ng/g	208.3 ng/g
	BDE-207	268.8 ng/g	132.7 ng/g	235.9 ng/g	181.5 ng/g	78.6 ng/g	213.9 ng/g
	BDE-208	16.7 ng/g	16.3 ng/g	22.1 ng/g	24.7 ng/g	20.5 ng/g	24.4 ng/g
	Total nona isomers	540.5 ng/g	268.1 ng/g	611.2 ng/g	568.3 ng/g	206.3 ng/g	446.6 ng/g
	Ratio to BDE-209	1.1	0.5	0.3	0.3	0.2	0.2
Octa-BDE isomers	BDE-196	141.6 ng/g	50.6 ng/g	100.9 ng/g	75.3 ng/g	91.4 ng/g	95.0 ng/g
	BDE-197	100.0 ng/g	44.1	89.5	70.5	nd	66.8
	Total octa isomers	241.6 ng/g	94.7 ng/g	190.4 ng/g	145.8 ng/g	91.4 ng/g	161.8 ng/g
	Ratio to BDE-209	0.5	0.2	0.1	0.1	0.1	0.1
Hepta-BDE isomers	BDE-191	nd	71.1 ng/g	nd	61.3 ng/g	nd	nd
	BDE-183	83.4 ng/g	46.0 ng/g	nd	nd	29.7 ng/g	60.1 ng/g
	Total hepta isomers	83.4 ng/g	117.1 ng/g	nd	61.3 ng/g	29.7 ng/g	60.1 ng/g
	Ratio to BDE-209	0.2	0.2	0.0	0.0	0.0	0.0
Hexa-BDE isomers	BDE-138	76.5 ng/g	nd	nd	nd	nd	58.8 ng/g
	BDE-156	65.2 ng/g	33.8 ng/g	nd	nd	nd	46.3 ng/g
	BDE-153	nd	nd	nd	nd	21.9 ng/g	44.0 ng/g
	BDE-154	32.1 ng/g	nd	nd	nd	12.8 ng/g	23.5 ng/g
	Total hexa isomers	173.8 ng/g	33.8 ng/g	nd	nd	34.7 ng/g	172.6 ng/g
	Ratio to BDE-209	0.3	0.1	0.0	0.0	0.0	0.1
Hepta-plus Hexa-BDE isomers	Total Hexa + Hepta	257.2 ng/g	150.9 ng/g	0.0 ng/g	61.3 ng/g	64.4 ng/g	232.7 ng/g
	Ratio to BDE-209	0.5	0.3	0.0	0.0	0.1	0.1

(adapted from Table S5 of Huang et al.[26])

These tables provide information on the ratios of the different degradation products to the starting substance. They also provide evidence for production of hepta isomers. Some key points are:

- There is considerable evidence of substances of concern in both shoots and roots, relative to the levels of untransformed deca-BDE present. Some of these plants are directly eaten by animals/man; thus this is a route of direct exposure.
- In roots, ratios to the deca-BDE present ranging from 10 to 50% for octa isomers and 10 to 30% for the combined level of hepta- plus hexa-BDE isomers are observed.
- In shoots, ratios to the deca-BDE present ranging from 10 to 50% for octa and 10-60% for hepta- plus hexa-BDE.

MESOCOSM STUDY

A Canadian study using mesocosms (10 M diameter) has been reported online (http://www.ontarioaquaculture.com/files/ELARES2008.pdf). This appears to be a well conducted study that is still in progress. It is a large scale study in semi-natural conditions in lakes. Until it is complete and published it is not possible to fully evaluate the contributions that this study will make to the question being addressed in this review, but a few preliminary conclusions may be drawn from the results presented to date.

In contrast with the paper of Huang et al.[26], which addressed a soil/plant system, this study used mesocosms sited in a lake environment. Based on the preliminary results reported to date, analysis of the sediments indicated the following:

- DecaBDE breakdown products were observed in surface sediments as early as one month after deca-BDE addition in all experiments.
    - The major products were nona-BDEs (BDE-206, 207 and 208).
    - Octa-BDEs were minor products at one and eight months
    - Tri-, tetra- and penta-BDEs were also observed in the medium and high deca-BDE mesocosms, but near or at detection limits in the controls.
- The proportion of deca-BDE in total PBDEs declined slowly from 99% at one month to 89% at 12 months in one experiment but remained at ~ 96% after one month and four months in a second experiment.
- Production of penta-, hexa-, hepta- and octa-BDEs was ~ 10 xs higher in the second experiment than the first while the production of nonaBDEs was similar in both experiments.
- The congener pattern was similar in both experiments. BDE-205 and BDE-194 were not detected, while the predominance of BDEs 206 and 207 and BDEs 196, 197, 200 and 201 suggests progressive loss of Br from positions 5 and 6.

Overall, deca-BDE breakdown products were observed in surface sediments as early as 1 month after decaBDE addition in all experiments; however, evaluation of the levels and actual isomers formed awaits more detailed reporting of the results.

In a communication from this team of researchers the ACHS has been informed:

1. We intend to prepare manuscripts on the results of the mesocosm experiment this winter. One manuscript will present temporal trends in PBDE concentrations in environmental compartments (water, particles, sediments, and periphyton) and will include a mass balance for each mesocosm. A second manuscript will focus on the bioaccumulation of DecaBDE and its breakdown products by fish (yellow perch) and

their prey items (composite zooplankton and 2 taxa of benthic invertebrates). The latter manuscript will likely also contain data on toxicity end-points for yellow perch.

2. We have been looking for BDE-126 in the food web, and detected this congener in some yellow perch from the "medium" and "high" mesocosms collected in 2009 (after 3 months of DecaBDE exposure), but in none of the fish from the "control" or "low" mesocosm. It was also not detected in zooplankton (collected 1 month after DecaBDE application). Unfortunately, BDE-126 has not been examined in water and sediments, but this discrepancy between the two labs has been pointed out previously and will hopefully be remedied in the near future.
3. For your information, we are following up on the mesocosm experiment this year with a series of smaller in situ experiments to probe the mechanisms of debromination. Sediment cores will be dosed with DecaBDE and incubated in our study lake under various condition (light vs. dark, biotic vs. abiotic). We will also be comparing the relative rates of DecaBDE debromination in pelagic (dark/anoxic/high carbon) vs. littoral sediments (light/oxic/low carbon).

BIOMAGNIFICATION AND BIOACCUMULATION

The potential of deca-BDE to undergo biomagnification and bioaccumulation is reviewed in Appendix 2.

ENVIRONMENTAL MONITORING STUDIES

The available data show that deca-BDE occurs widely in indoor and outdoor air and dust. The study of Wilford et al.[27] Is particularly relevant, since it provides evidence that the deca-BDE found in indoor air is predominantly associated with particles formed by abrasion of textile articles, indicating the importance of this emission source. Whilst the congener profile found in this study is suggestive of debromination of deca-BDE to nona-BDEs in dust samples, Stapleton and Dodder[4] provide further evidence that hepta-BDEs can be formed by photodegradation of deca-BDE adsorbed onto dust. This appears to be an environmentally relevant degradation mechanism. Such dust may be considered as a significant, uncontrollable and long-term diffuse source of deca-BDE in the environment, and although it is difficult to determine the extent of exposure of such dust to light, such exposure is likely to occur, particularly in the atmosphere, etc. It is therefore considered that deca-BDE on indoor dust can be a source of hepta-BDE congeners in the wider environment.

Currently, there is a 10-year programme (first sampling year 2005) sponsored by the Bromine Science and Environmental Forum (BSEF) to monitor long-term trends of deca-BDE in various environmental matrices—sewage sludge, sediment, air and birds' eggs. Annual or biennial samples are taken. Sludge from 12 EU sites (predominantly in the UK and the Netherlands) is collected at each site over a one week period while sediment (top 2 cm layer) is collected as four composite samples from each of 10 EU sites; each composite sample consists of nine sub-samples from an area of approximately 100 m^2. Air sampling (particulate plus vapour phase) has been conducted at a single semi-rural site (94 m above sea level) in north-west England. Biotic sampling has involved analysing the glaucous gull (*Larus hyperboreus*) eggs from Bear Island (Bjørnøya) in northern Norway and sparrow hawk (*Accipiter nisus*) eggs from the UK.

Currently, there have been insufficient years of sampling to date to evaluate long-term temporal trends in deca-BDE in any of the environmental matrices. This analysis will be carried out at the end of the project although it is unclear what power the analysis will have to detect change. Where limited comparisons have been made to date between years in which samples have been collected, there was either little difference in deca-BDE concentrations or no consistent pattern of change within or across different matrices[28]. There is marked spatial variation in deca-BDE concentrations however, with concentrations more than an order of magnitude higher in UK than in Dutch sludge, and sediment deca-BDE concentrations varying by some three orders of magnitude between estuaries; this may in part be influenced by the amount of Total Organic Carbon in the sediment. Deca-BDE concentrations also vary between bird species and are higher in the sparrow hawk than the glaucous gull eggs[29]. This may be due to a number of abiotic and biotic factors. Deca-BDE concentrations are reported to have increased between 1996 and 2006 in peregrine falcon eggs from the North-eastern U.S.[30].

Concentrations of selected lower brominated congeners have also been measured in some of the matrices. BDE-126 (3, 3', 4, 4', 5-pentabromodiphenyl ether) is reported to be formed by abiotic degradation of decaBDE under anaerobic reducing conditions and so may be a marker of abiotic degradation. This congener is not expected to be present in commercial products. It was detected in sediment and sewage sludge samples collected in 2007 at levels up to about 0.3 µg/kg and 0.1 µg/kg dry weight respectively[28]. This is an important finding because it suggests that this and presumably other penta-BDEs and intermediate higher PBDE congeners are being formed in the environment (though not necessarily in sediment or sludge itself) as a result of degradation of deca-BDE. BDE-126 has also been reported in two species of fish [31], presumably as a result of uptake from sediment or sludge.

A second congener, BDE-202 (2,2',3,3',5,5',6,6'-Octabromodiphenyl ether) is also not found in commercial products and is thought to be a potential marker for debromination of deca-BDE, potentially through photolytic[4,32] and metabolic[33] mechanisms. BDE-202 has been found in sediments[34] and a number of wildlife species[30,35,36], although the presence of BDE-202 may result from dietary ingestion of the debrominated congener and is not evidence of debromination by those particular species.

In summary, monitoring studies to date are now producing accumulating (though still indirect) evidence that the debromination of deca-BDE, previously only predicted by laboratory experiment, is actually occurring in the environment. Debromination may occur abiotically, *in vivo* in some organisms, and/or debromination products may be directly bioavailable and assimilated by organisms. Monitoring data to determine temporal changes over time in debromination products are confounded by changes in use patterns and product purity, and by other commercial PBDE sources, known to include penta-BDE and octa-BDE. There do not appear to be data reporting long-term trends in the suggested degradation-specific congeners (BDE-126, 202), although the industry monitoring study may produce some such data for sludge and sediments in due course.

Legacy and imported products are less well understood, and probably of lower purity. Possible derivatives such as hydroxylated compounds are also poorly characterised.

METABOLISM

VERTEBRATES

This section focuses primarily on the rat, this being the species in which the majority of detailed studies have been conducted, but data from experiments on birds and fish are also considered.

MAMMALS

The primary aim of this section is to review the potential for metabolic debromination of deca-BDE in mammals. Rather than focussing exclusively on debromination, and in order to place the limited information available in context, this section considers the bioavailability and disposition of deca-BDE along with information evidence concerning its routes of metabolism and excretion.

Disposition of deca-BDE

Deca-BDE is unlikely to undergo uptake via passive diffusion in the small intestine since it is highly hydrophobic and has a molecular weight of 959 (well above the cut-off for passive diffusion, which is about 300). Indeed, up to 90% of a dose of deca-BDE is recovered from the faeces following dietary or oral administration. This has been taken to indicate that deca-BDE is poorly absorbed[37]; however, the fact that the majority of the material recovered from faeces is in the form of metabolites suggested that deca-BDE may be absorbed from the gut, metabolised in the liver and excreted via the faeces. Intravenous (i.v.) dosing studies tended to support this conclusion, since the majority (74%) of a single i.v. dose of deca-BDE (1.07 mg/kg) is found in the faeces and 65% of this is in the form of metabolites[37]. Deca-BDE may be metabolised in the intestinal contents by the gut microflora as well as by endogenous xenobiotic metabolising enzymes in the liver.

One problem in evaluating the absorption and disposition of deca-BDE is that it is poorly soluble in conventional vehicles. Special formulations have therefore been developed to permit studies on the intestinal absorption of deca-BDE. The use of a soya phospholipone:Lutrol (16:34)/water based formulation allowed the bioavailability of deca-BDE to be optimised and its metabolism and disposition to be evaluated[38]. Following an oral dose of deca-BDE (3 μmol/kg), 90% was excreted via the faeces after 3 or 7 days, 9% was retained in the tissues and less than 0.1% was excreted in the urine. Bile duct cannulation revealed that 10% of the administered dose was found in the bile after 72 hours, indicating that at least this much deca-BDE was absorbed. The main sites of retention were the liver and plasma while little radioactivity was detected in adipose tissue. This is an unusual observation for such a lipophilic compound (deca-BDE has a log K_{OW} of 12.1) and has not yet been explained, although it is consistent with the low volume of distribution of deca-BDE[39]. One possibility is that deca-BDE or its metabolites bind(s) extensively to proteins in the liver and/or plasma.

The results of these studies indicated that deca-BDE was excreted into the faeces both in the bile and via other pathways, possibly involving extrahepatic metabolism and/or active transport mechanisms. One possibility is that deca-BDE could undergo first pass metabolism by cytochrome P450 (CYP) enzymes in the liver and/or wall of the small intestine[39]. This hypothesis is consistent with the observation that deca-BDE undergoes covalent binding in the

gut wall. The presence of deca-BDE metabolites in the faeces may also be a consequence of metabolism in the intestinal contents by the gut microflora.

Routes of metabolism

The following metabolites of deca-BDE have been identified in rats[38-41]:

- Nona and octa-BDEs (BDE-201, BDE-202, BDE-206, BDE-207 and BDE-208) and their methoxy/hydroxylated derivatives.
- Methoxy/hydroxylated hepta-, hexa- and penta-BDEs (at least six different metabolites). These metabolites are believed to be guaiacol structures (i.e. the methoxy and hydroxy groups are on adjacent carbon atoms).
- Various polar metabolites (detected in urine and intestinal contents but not faeces). These are consistent with the formation of hydrophilic conjugugates which are subsequently deconjugated by the gut microflora.

The dominant metabolites in rat plasma appear to be hydroxylated nona- and octa-BDEs. The pattern of metabolites observed is considered to be consistent with the hypothesis that the initial step in deca-BDE metabolism is enzymatic debromination starting at the *meta* position[38,39,41] although the maximum number of bromine atoms which can be removed by this process is unclear and no confirmatory evidence is available. Metabolic debromination may be followed either by arene oxidation and dihydrodiol formation or by two consecutive oxidations and methylation by catechol O-methyltransferase. However, no experimental evidence is available in support of either of these suggestions. The formation of reactive metabolites such as quinones is also considered possible[38], but again no evidence has been obtained for this route.

Hepta-BDE has been reported in rat tissues following dosing by gavages or via the diet[41,42]. However, the methods used were unable to identify hydroxylated metabolites so it is unclear whether or not the metabolite detected was hydroxylated, and the level detected was too low to allow its origin to be determined. A subsequent study tentatively suggested the presence of hydroxylated hepta-BDE in pregnant rats following oral dosing, but the peak detected was also consistent with octa-BDE[40].

The terminal elimination half life of deca-BDE in rats has been estimated to be approximately 2.5 days[39] following oral administration in DMA, PEG400 and water (4:4:1) (although another study which used dietary administration indicated a much longer half life (75.9 days[42]). The study of Sandholm et al.[39] Indicated an oral bioavailability of 26% for deca-BDE. The plasma levels of phenolic metabolites in rats 3-7 days after oral dosing exceeded that of the parent compound, indicating that overall exposure to the phenolic metabolites is greater than that to deca-BDE itself. There is some evidence that the half life of deca-BDE metabolites increases as the degree of bromination decreases from deca to nona to octa[42].

The formation of nona and octa-BDEs by *meta* (nona-BDEs) and *meta/para* (octa-BDEs) debromination could occur as a result of reductive debromination by microflora in the gastrointestinal tract.

Induction of xenobiotic metabolising enzymes

While the main concern of this statement is the potential for deca-BDE itself to undergo metabolic debromination, it is important to note that deca-BDE has the capacity to induce the

expression of xenobiotic metabolising enzymes, consistent with its propensity to induce liver enlargement. Early studies[43,44] suggested that deca-BDE might be a weak Phenobarbital-like inducer of cytochrome P450 (CYP) expression since it can induce the expression of CYPs of the steroid and barbiturate inducible families. More recent studies, which take advantage of the identification of the nuclear receptors responsible for CYP induction, have indicated that deca-BDE is a weak pregnane X receptor (PXR) agonist[45]. It may also interact with the constitutive androgen receptor (CAR)[41,46]. This may be of relevance when considering the potential environmental risks associated with deca-BDE as the compound may be able to induce its own metabolism (possibly including debromination) as well as that of other potential toxicants.

BIRDS

Only limited data are available concerning the potential for metabolic debromination of deca-BDE in birds.

In a study in which European starlings (*Sturnus vulgaris*) were exposed to deca-BDE over 76 days by means of a silastic implant [47], nona and octa-BDEs (BDE-197, BDE-206, BDE-207 and BDE-208) were markedly elevated in liver and muscle of exposed birds compared with controls. Hexa-BDE (BDE-153) and hepta-BDE (BDE-183) were also present, but was only elevated ~2 fold in exposed birds. This paper provides circumstantial evidence that deca-BDE could be debrominated, at least to octa and nona-BDEs, in starlings but no definitive metabolism results are presented.

A recent study[35] claims to provide evidence for debromination of deca-BDE in Californian peregrine falcon (*Falco peregrinus*) eggs. This study examined the homologue patterns of hepta, octa and nona-BDEs found in eggs collected in the environment. Various PBDE congeners were detected, including two nona-BDEs (BDE-207 and BDE-208), an octa-BDE (BDE-202) and an unidentified hepta-BDE. However, it is limited in that the composition of the material to which the birds had been exposed was uncharacterised. Furthermore, the eggs had been collected over a period of 22 years (1986-2007) and were all eggs that had failed to hatch (having either been found addled during collection or failed to hatch during captive incubation). The data obtained may not, therefore, be representative of viable eggs. Overall, this study does not provide strong evidence for metabolic bromination in peregrine falcons.

FISH

Several studies have examined the dietary uptake and biotransformation of deca-BDE and found that fish fed food spiked with deca-BDE were found to accumulate lower brominated congeners[48-51]. The assimilation and debromination of deca-BDE varied among the three fish species examined, which included rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*) and lake trout (*Salvelinus namaycush*). Common carp accumulated no deca-BDE in their tissues but they did accumulate one penta, three hexas, two heptas and one octa-BDE congener that appeared to results from debromination of deca-BDE. In two separate studies on rainbow trout accumulation of PBDE was found although the uptake was less than 1% in both studies. Both studies observed an increase in hexa-, hepta-, octa- and nona-BDE congeners over time that comprised a higher percentage of the PBDE body burden relative to deca-BDE body burden.

In the study of Kirkegaard et al.[51], juvenile rainbow trout were fed cod chips spiked with deca-BDE. Deca-BDE was not detected in the fish, but BDE-47, -99, -153, and several non-specified hexa- to nona-BDEs were reported. Their concentrations in liver and muscle increased with length of exposure. BDE-153, -154 and an unidentified octa-BDE were not detected in the original deca-mixture, indicating likely transformation of deca-BDE.

Tomy et al.[49] Studied the uptake, by juvenile lake trout, of twelve tetra- to hepta-BDEs plus deca-BDE from spiked commercial fish food. Three lower brominated PBDE congeners (unknown penta- and hexa-BDE, and BDE-140) appeared to be biotransformed in the exposed fish and the authors hypothesised that debromination of deca-BDE was a potential explanation. They suggested that the structural similarity of BDEs to thyroxine (T4) could mean that deiodinase enzymes were debrominating higher brominated PBDEs to lower brominated PBDEs. However, they also suggested that the process may involve other enzyme pathways such as cytochrome P450 1A and 2B (i.e., Phase I enzymes), which are known to hydroxylate aromatic contaminants such as polychlorinated biphenyls or polycyclic aromatic hydrocarbons. The authors concluded that the degree biotransformation, especially for deca-BDE, was likely to vary considerably between species, leading to high potential interspecies variability in bioaccumulation.

The best evidence for metabolic debromination of deca-BDE in fish is found in studies by Stapleton et al.[48,50]. In the first of their studies, juvenile carp were fed deca-BDE (>98%) spiked food for 60 days. At the end of this study deca-BDE was not detected in the carp tissues, however seven other congeners were observed and accumulated over time. These were:

- One penta-BDE (“penta-1”)
- Three hexa-BDEs (BDE-154, BDE-155 and “hexa-3”)
- Two hepta-BDEs (“hepta-1 and “hepta-2”)
- One octa-BDE (“octa-1”)

The amounts detected exceeded those which could have accumulated as a consequence of selective uptake of contaminants from the food. Furthermore, penta-1 increased over time while octa-1 decreased, even during the withdrawal period. This suggests the possibility of ongoing debromination of body stores of PBDEs. This study provided good circumstantial evidence for metabolic debromination of deca-BDE in fish although it did not demonstrate metabolism directly.

A follow-up study addressed this omission and extended the analysis to another species. In this study, rainbow trout (n=45) were exposed in the laboratory to deca-BDE (98% pure) via the diet for 5 months[50]. Deca-BDE accumulated in the liver, suggesting that the liver could act as a sink for this compound, and was also detected at high levels in serum, possibly as a result of tight binding to serum proteins. The debrominated compounds detected in trout tissues were:

- Three nona-BDEs (BDE-206, BDE-207 and BDE-208, although BDE-206 did not accumulate with time).
- Six octa-BDEs (the major forms being BDE-201 and BDE-202)
- Four hepta-BDEs (the major form being BDE-188)

The lower congeners were found at concentrations exceeding those which could have accumulated from impurities in the food itself and debrominated products accounted for approximately 73% of the total PBDE burden in the carcasses. Nona-BDEs (primarily BDE-207 and -208) accounted for 26% of the burden in serum with only minor amounts of octa-BDEs present, and untransformed deca-BDE accounting for the remainder (approximately 68%). In the liver, the burden was primarily deca-BDE with only a small fraction of lower brominated PBDEs (primarily nona-BDEs). The predominance of BDE-202 as a product of deca-BDE debromination was similar between rainbow trout (observed here) and carp from the previous study.

It should be noted that hydroxylated and covalently bound metabolites were not sought in this study, so it is difficult to compare the results with those obtained in the rat and further oxidative metabolism of debrominated metabolites cannot be excluded.

To determine whether the observed debromination was a result of metabolism by the fish, liver microsomes were prepared from both carp and rainbow trout and incubated with deca-BDE. The metabolic activity of the microsomes used was verified by measuring ethoxyresorufin O-deethylase and the incubation mix was supplemented with NADPH (100 µM), although no NADPH regenerating system was provided. The metabolites identified were as follows:

- In rainbow trout liver microsomes, nona- and octa-BDE congeners.
- In carp liver microsomes, hexa-, hepta-, octa- and nona-BDEs. The nona-BDEs did not accumulate, consistent with the absence of nona-BDEs in fish exposed *in vivo* via the diet. Two hexa-BDE congeners, BDE-154 and BDE-155 were identified and accounted for ~30% of the added deca-BDE.

These results are consistent with metabolic debromination of deca-BDE, which could be catalysed by deiodinases or possibly in an atypical CYP-mediated reaction. Stapleton et al. concluded that their results supported the hypothesis that deiodinase enzymes were catalyzing debromination of deca-BDE; however, they also cautioned that it was not possible to rule out the concurrent or alternative action of CYP enzymes. The species difference observed tends to support the possibility that deiodinases are involved, since carp express higher levels of deiodinase activity than do other species. Stapleton's work also indicates that removal of bromine atoms occurs preferentially from the meta- or *para*-substituted positions.

These data demonstrate that fish are able to debrominate deca-BDE down to hexa-BDE congeners. They do not, however, provide any clear information about the final product of metabolism since the possible products of oxidative metabolism and the potential for covalent binding to proteins were not considered.

Other studies on the potential for metabolic debromination of deca-BDE have addressed biotransformation in zebra fish (*Danio recio*[52]) and lake whitefish (*Coregonus clupeaformis*[53]), but have only generated circumstantial evidence. The study of Nyholm et al.[52] Cannot be used to draw conclusions about the debromination of deca-BDE since the fish were exposed to a mixture of eleven brominated flame retardants, including a hepta-BDE (BDE-183) as well as two tri-BDEs. The study of Kuo et al.[53] Evaluates the uptake and potential effect on juvenile lake whitefish (*Coregonus clupeaformis*) of deca-BDE. In this study Lake Whitefish were fed deca-BDE at 4 nominal concentrations (control, 0.1, 1 and 2 µg/g diet) for 30 days. Liver and

carcasses were analysed for 11 PBDEs. Four congeners (BDE-206, -207, -208 and -209) were detected. Concentrations of all congeners from the 1 and 2 µg/g groups were higher in livers than carcasses, indicating the liver was the primary organ of deca-BDE accumulation. One congener, BDE-206, was thought to be a major metabolite from deca-BDE bromination. This *in vivo* study indicated that deca-BDE was debrominated into lower PBDE congeners and that exposure to 2 µg/g may have affected fish growth.

From these laboratory studies it is apparent that deca-BDE can undergo metabolic debromination in at least some fish species. Hence, once deca-BDE is released to the environment it may encounter conditions conducive to debromination. La Guardia et al [36] have examined the potential for *in vivo* debromination of deca-BDE in aquatic organisms inhabiting the receiving environment of a wastewater treatment plant (WWTP) located in Roxboro, North Carolina, which, based on releases reported by industry to the US EPA's Toxics Release Inventory, was determined to receive wastewater from a large plastics manufacturing facility. The PBDE congener profile was tracked from the WWTP effluent to the receiving environment sediments and to biota in order to evaluate whether significant debromination was occurring. In 2002, samples of wastewater sludge, sediments and biota (sunfish, *Lepomis gibbosus*, creek chub; *Semolilus atromaculatus* and a crustacean crayfish *Cambarus puncticambarus sp*) were collected and in 2005, samples of wastewater sludge, sediments and biota (sunfish only) were collected.

A total of 23 PBDE congeners were detected in the biota samples. Of these, deca-BDE was only detected in 2002 samples of sunfish (2880 µg/kg lipid) and crayfish (21 600 µg/kg lipid). The much higher concentration in crayfish was attributed to the sediment-association of this species and the authors speculated that crayfish could form a link from sediments to pelagic organisms. The authors also speculated that the lack of detected deca-BDE concentrations in chub could be due to an enhanced ability of this species to metabolize deca-BDE. Chub are closely related to carp, which have previously been demonstrated to have an enhanced capability to debrominate deca-BDE[48]. The chub composite contained 3 nona-BDE, 4 octa-BDE and 2 hepta-BDE, of these two octa- (BDE-201, -202) and 3 hepta- (BDE-188, -184, -179) congeners was not detected in either sludge or sediment samples, suggesting biotransformation of these homologues. Chromatograms of the laboratory exposed carp in Stapleton et al [48] and the chub from the La Guardia et al.[36] Study exhibited comparable hepta- through deca-congener patterns. PBDEs present identified as one octa (BDE-202) and two hepta (BDE-188 and -179). These were not detected in the sediment or sludge. Based on these findings, the authors concluded that deca-BDE is bioavailable in natural environments and could undergo metabolic debromination in the field, resulting in bioformation of lower brominated PBDEs.

With respect to information about the final product of metabolism from debromination of deca-BDE in fish (i.e. whether the possible products of oxidative metabolism and the potential for covalent binding to proteins have been considered); from the observations of Stapleton (personal communication), lower brominated PBDEs are primarily detected and no hydroxy-BDEs are seen in fish. However, it is speculated that there could be covalent binding to proteins although it is much harder to detect and measure these. Stapleton does not observe a complete mass balance in her metabolism studies, which does suggest the formation of other metabolites and/or covalent binding to proteins. Based on the findings of other studies with rats (e.g. Morck et al.[38]), it is possible that deca-BDE could also be transformed by fish to hydroyxlated and/or

methoxylated debrominated congeners. If these congeners were formed in fish, the reported net uptake of neutral BDEs would underestimate the actual total uptake of deca-BDE. This could explain the much lower absorption efficiency observed for fish relative to that observed with rats, where both neutral and hydroxylated/methoxylated BDEs were analyzed. Furthermore, if metabolites other than hepta-, octa- and nona-BDEs are being formed and persisting in the fish, then deca-BDE accumulation studies which measure only neutral PBDEs would underestimate the total accumulation potential of deca-BDE-related compounds.

Currently it is not known which enzyme system is catalysing the debromination and hence it is almost impossible to determine which fish species can and cannot debrominate deca-BDE. Through the studies of Stapleton et al. and through perusal of the research literature (Stapleton, pers. com.) it is believed that almost all Cyprinid fish (e.g. carp, minnows, zebra fish, etc) can debrominate deca-BDE very well. There is also evidence to support debromination of deca-BDE in American eels, sculpins, and rainbow trout. The current hypothesis is that these fish have higher expression and activity of deiodinase iso-forms that have a binding affinity for PBDEs. Work is currently ongoing (Stapleton, personal communication) exploring this hypothesis in addition to comparing the PBDE debromination potential among carp, rainbow trout and salmon. Debromination of deca-BDE is seen in all three species although the activity is significantly higher in carp. Those cyprinid fish are very efficient at debrominating PBDEs for some reason still to be established.

INVERTEBRATES

The PBDE-transformation potential of invertebrates appears not to have been studied to any great extent and there are few data suggesting that invertebrates in aquatic systems (e.g. shrimp, shellfish, worms etc) can debrominate deca-BDE. It is unlikely that invertebrates are able to debrominate deca-BDE given that invertebrates typically have less developed biotransforming enzymes than vertebrates (Stapleton, pers.com).

However, some circumstantial evidence has been presented recently. Klosterhaus and Baker [2] have investigated the mechanism controlling bioavailability of deca-BDE in a 28-day bioaccumulation experiment in which the marine polychaete worm *Nereis virens* was exposed to deca-BDE commercial mixture (90% deca-BDE) in spiked sediment, in spiked food or field sediment. Bioaccumulation from spiked substrate with maximum bioavailability demonstrated that deca-BDE accumulates in this species. Bioaccumulation depends on exposure conditions, however, because deca-BDE in field sediment did not accumulate (<0.3 ng/g wet weight, 28-d biota-sediment accumulation factor, BSAF, <0.001) despite high exposure concentrations of deca-BDE (>2000 ng/g dry weight). Of particular interest was the approximately 10 times higher bioavailability of BDE-208 compared with other congeners in the deca-BDE mixture. Higher bioavailability of BDE-208 compared with other congeners in the deca-BDE mixture was also observed in the sediment exposure. The author suggested that these results may indicate biotransformation of deca-BDE by *N. virens* to BDE-208 rather than higher bioavailability from sediment or exposed sediment. *N. virens* efficiently metabolise polycyclic aromatic hydrocarbons and may have a limited ability to biotransform polychlorinated biphenyls [2]; however, PBDE transformation potential in this species (or any other invertebrates) has not been studied. The mechanisms responsible for limited accumulation of deca-BDE were suggested to involve characteristics of the sediment matrix and low transfer efficiency in the digestive fluid. These results are consistent with a similar 28 day study by Ciparis and Hale[54] in

which deca-BDE was not detected in the freshwater oligochaete worm *Lumbriculus variegates* exposed to biosolids but was minimally detected, though below quantification limits, in worms containing similar concentrations of deca-BDE (300 ng/g dry sediment). In contrast to these studies, earthworms living in deca-BDE contaminated soils accumulated deca-BDE, which may indicate that deca-BDE is more bioavailable to terrestrial food webs[55].

To conclude from the evidence presented so far, it seems that invertebrates would have a very low potential to debrominate deca-BDE. In addition, no information is currently available for deca-BDE regards to the acute toxicity towards invertebrates. A long-term *Daphnia* test has been carried out using octaDBE (European Union Risk Assessment, 2002). No effects on survival, reproduction or growth were seen over 21 days as concentrations up to 2 microgram/L (solubility limit). Taken as a whole , it is clear that the aquatic toxicity and bioaccumulation potential of penta, octa and deca-BDEs decreases with increasing bromination and therefore it is unlikely that deca-BDE will show toxic effects to invertebrates because its solubility limit.

POTENTIAL TOXICITY OF DEGRADATION PRODUCTS

In the past, deca-BDE has given rise to few issues in relation to mammalian toxicity; any case for identifying deca-BDE as an SVHC on the basis of potential mutagenic, carcinogenic or reproductive toxicity would have to be based upon its capacity to undergo metabolic debromination to penta, hexa, hepta or octa-BDE. It should, however, be noted that deca-BDE, like other PBDE congeners, has been reported to induce developmental neurotoxicity in rodents. While this property alone would not be sufficient to identify deca-BDE as a SVHC, young children would be at potential risk. A summary of the data is therefore provided in support of general discussions around the potential hazards associated with exposure to deca-BDE.

TOXICITY OF DECA-BDE ITSELF

Deca-BDE is generally considered to be of low toxicity in mammals. It has minimal adverse effects in two-year studies in the rat although it does induce liver enlargement in rats following short term administration[37,43,44] and this is associated with slight centrilobular hypertrophy consistent with the possibility that deca-BDE is a hepatic enzyme inducer[41].

TOXICITY OF LOWER BROMINATED CONGENERS

The potential toxicity of lower brominated congeners of diphenyl ether has been studied thoroughly. A summary was provided to ACHS prior to the initiation of its discussions in March 2010 and is reproduced in Appendix 3.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF DECA-BDE AND LOWER BROMINATED CONGENERS

One possible breakdown product of deca-BDE is octa-BDE. This substance, which has itself been used as a flame retardant but whose use is now restricted, has been tested in some detail and is the subject of an EU Risk Assessment[5]. Octa-BDE is not mutagenic under standard regulatory

[5]http://ecb.jrc.ec.europa.eu/documents/Existing-Chemicals/RISK_ASSESSMENT/REPORT/octareport014.pdf).

testing conditions. No data indicating any carcinogenic effects have been reported, although carcinogenicity cannot be excluded as the available studies were of insufficient duration to provide an unequivocal answer on this point. Octa-BDE has, however, been identified as a reproductive toxin. Its main effect is developmental although it also has adverse effects on fertility (Repr. Cat 2 R61: may cause harm to the unborn child; Repr. Cat 3 R62: possible risk of impaired fertility).

It should be noted at the outset that, as highlighted in the EU Risk Assessment for octa-BDE (p65, Section 3.2): "The interpretation of the toxicity data for commercial octabromodiphenyl ether is not straightforward as the substance is a mixture containing significant amounts of lower brominated diphenyl ethers, notably hexabromo-diphenyl ether.....".

EFFECTS ON FERTILITY

No specific studies addressing the potential effects of octa-BDE on fertility have been carried out and no histological changes in reproductive organs (testes, prostate, ovaries or uterus) have been identified during standard toxicology tests up to a dose of 10,000 ppm in the diet. However, two incidental observations have led to the conclusion that octa-BDE may have an adverse effect on fertility:

- Reversible enlargement of the testes is observed in some, but not all, studies.
- Complete absence of corpora lutea has been observed in 3/10 females dosed at 202 mg/m3 by inhalation. This was associated with a decrease in the weight of the ovaries and is very unusual in rats of the age used (20 weeks). Octa-BDE was allocated a hazard classification of Cat 3 R62 on the basis of these data.

DEVELOPMENTAL TOXICITY

The developmental toxicity of commercial octa-BDE formulations has been tested in rats (three studies) and rabbits (one study). Key facts from these studies are summarised in Appendix 4.

As noted by the EU risk assessors, it is difficult to interpret these data because the commercial formulations tested actually contained <40% octa-BDE. It is possible, therefore, that the developmental toxicity observed was due to other constituents of the mixture. In particular, hepta-BDE was the major constituent of each of the formulations tested. Unfortunately no reproductive toxicity is available for hepta-BDE itself because it is not deliberately used in commercial formulations (although the presence of high levels of this material in commercial octa-BDE products suggests that its toxicity should be evaluated). It is impossible to be sure whether the observed reproductive toxicity of commercial "octa-BDE" is actually a characteristic of octa-BDE itself, although the fact that the reproductive effects of FR-1208 (which contains less octa-BDE and more hepta-BDE than the other products tested) were less marked than those of DE-79 and Saytex 111 provides circumstantial evidence that octa-BDE might be the material responsible. This observation also suggests that the reproductive effects of octa-BDE may exhibit a threshold, although this conclusion can only be drawn extremely cautiously.

With respect to potential reproductive effects of deca-BDE arising as a consequence of debromination to octa-BDE, no firm conclusion may be drawn. There is no doubt that commercial octa-BDE formulations exhibit reproductive toxicity in at least two species, and the

material has been classified accordingly. However, the possibility that this is due to components of the mixture other than octa-BDE itself has not been tested. The possibility that deca-BDE could be debrominated to octa-BDE, and consequently exhibit reproductive toxicity, cannot be entirely excluded. Furthermore, it is possible that reproductive toxicity could occur as a result of further debromination to hepta-BDE. Again, this has not been tested. However, given the high doses required to induce reproductive toxicity it would appear unlikely that the amounts of octa-BDE and/or hepta-BDE generated from deca-BDE, either *in vivo* or in the environment, are sufficient to constitute a significant risk in this context.

DEVELOPMENTAL NEUROTOXICITY

There is evidence that polybrominated diphenyl ether may exert neurotoxic effects during the early stages of postnatal development. These effects are observed following dosing of mice during the neonatal brain growth spurt. This phase of development, which occurs during the first few weeks of postnatal life in rodents (peaking on day 10), corresponds to the third trimester of pregnancy and first two years of postnatal life in humans, so it overlaps with the period covered by developmental reproductive toxicology tests. The changes which occur during this period include[56,57]:

- Maturation of dendritic and axonal outgrowths.
- Establishment of neuronal connections.
- Synaptogenesis.
- Proliferation of glial cells.
- Myelinisation.
- Acquisition of motor and sensory abilities.
- Peak of spontaneous motor behavior
- Rapid development of the cholinergic transmitter system.

The effects in early adulthood of administering PBDEs during the murine neonatal brain growth spurt have been tested using two types of behavioural tests, as follows:

- Spontaneous behavior test: This measures motor activity over a period of 60 minutes after placing the animal in an unfamiliar environment. It evaluates exploratory behavior in terms of locomotion, rearing and total activity.
- Swim maze test: This measures learning ability in terms of improvements over 5 days in ability to locate a submerged platform.

Some studies have also measured other parameters such as cholinergic receptor status and protein expression in the brain.

The results of studies reported in the peer reviewed literature are summarised in Appendix 5.

Where adverse effects are reported, these have followed a similar pattern for all PBDEs. Treated mice exhibited a disruption of habituation when placed in an unfamiliar environment. (Habituation is defined as a decrease in locomotion, rearing and total activity over time in response to the diminishing novelty of the test chamber over the 60 minutes of the test). The treated mice were hypoactive during the first 20 minutes of the test but subsequently became hyperactive (i.e. at the beginning of the test they were less active than vehicle-treated controls

but during the last 40 minutes they were more active). In the case of the swim maze test, the observed adverse effects involved treated mice taking longer to be able to locate a submerged platform and being less able to improve this performance over time than control animals. While these effects are quite subtle and the mechanism by which they may be induced is unknown, similar behavioural impairments have been observed in aging humans so they may be of relevance to human hazard assessment.

On the basis of studies using deca-BDE, Viberg et al.[56] have drawn the conclusion that "Exposure of the mouse to deca-BDE during a defined period of development can give rise to irreversible changes in adult brain function". While this conclusion appears to be supported by the published reports available, it should be drawn with some caution for the following reasons:

- Almost all the data are found in papers from a single group (although some broadly supportive data have recently been published by other investigators).
- The effects observed are very specific as to time of exposure and have been identified using highly specialised testing methods, so their general applicability remains to be determined.
- Very little work has been done to try to establish a molecular or cellular mechanism for the effects observed.

A thorough study conducted to Good Laboratory Practice standards has not indicated any neurotoxicity following administration of deca-BDE to pregnant female rats and evaluation of the offspring. The key conclusions of the study are summarised as follows in its Final Report[6]:

"There was no evidence of maternal toxicity at any dosage level of decabromodiphenyl oxide evaluated in this study. Additionally, there were no effects on offspring survival and growth, or on any of the neurobehavioral parameters evaluated in this study. Normal patterns of habituation were observed at all relevant ages tested for both locomotor activity and auditory startle response. Therefore, under the conditions of this study, no evidence of developmental neurotoxicity was observed at any dosage level evaluated."

For ease of reference, the authors' summary of this study is presented in its entirety in Appendix 6.

Overall, it would appear that octa- and deca-BDE may exert adverse effects on neurological development, but these appear (on the basis of limited evidence) to be milder than those of penta- and hexa-BDE and do not justify classification for this effect under the Classification Directives/Regulation.

[6]An oral (gavage) developmental neurotoxicity study of decabromodiphenyl oxide in rats; Study number: WIL-635002, p27

SCIENTIFIC CONCLUSIONS

EVIDENCE FOR ENVIRONMENTAL DEGRADATION

TRANSFORMATION OF DECA-BDE IN THE ENVIRONMENT

It can be concluded that the likelihood, rates and potential transformation products of deca-BDE debromination will depend largely upon the medium in/on which it is present and the rate of various degradation processes (e.g. photodegradation, abiotic degradation, biodegradation) as follows:

Abiotic degradation

It appears that deca-BDE encapsulated in plastic does not undergo detectable photodegradation. However, significant debromination of deca-BDE will occur when exposed to sunlight and when limited particle shielding is present resulting in the formation of potentially hazardous by-products.

Definitive evidence has been presented that hepta-BDE can be formed by photodegradation of deca-BDE onto dust although it is impossible to infer how fast this occurred and what percentage of deca-BDE broke down because of the number of variables in each house (e.g. dust loadings, ventilation rates, changes in sunlight exposure). While the actual degree of sunlight exposure to household dust might be limited by window and shading, it has been noted that dust in cars would be subjected to much higher levels of sunlight, making debromination of deca-BDE in cars potentially significant.

Deca-BDE absorbed to dust or other dry minerals and particulates therefore appear susceptible to relative rapid transformation with half lives ranging from 76 minutes (deca-BDE sorbed to a thin film of kaolinite[17]) to 408 hours (deca-BDE sorbed to house dust[4]). Transformation appears to follow stepwise reductive debromination to form hexa to nona-BDE.

Biodegradation

The results of biodegradation studies are somewhat mixed. Early studies focused on deca-BDE mineralisation and these indicated very little, if any degradation, although it should be noted that these studies did not specifically examine debromination. In laboratory studies using activated sludge, Gerecke et al.[16,17] determined half-lives ranging from 693 to 1400 days depending on the presence/absence of primer, and identified debromination products as octa- and nona-BDEs. Recent soil biodegradation kinetic studies in aerobic and anaerobic soils confirm earlier results that showed little degradation of deca-BDE in both aerobic and anaerobic soils with digested or activated sludge[25]. In a separate study, He et al.[18] Observed complete transformation of deca-BDE to hepta- and octa-BDE over 2 months with one anaerobic culture (*Sulfurospirillum multivorans*) but negligible degradation with other anaerobic cultures. This study is corroborated by other studies providing evidence of significant variability in the extent to which different microbial cultures are able to degrade certain congeners of PBDEs. Hence, half-lives of deca-BDE in natural sediments vary by orders of magnitude in different experiments from 40-60 hours[12], 150 days[15], to 14 years[1]. Zhou et al.[23] also provide evidence of significant (42.2%) and rapid (10 days) degradation of deca-BDE by white rot fungi.

Monitoring studies of WWTP sludge provide little evidence of deca-BDE debromination in WWTP, possibly because the residence time in WWTP is too short for significant debromination to be observed. A more recent co-solvent biomimetic system and an anaerobic sediment microcosms study by Tokarz et al.[1] have demonstrated that reductive debromination of deca-BDE at decreasing rates with decreasing bromination does occur and identified at least 12 degradation products of deca-BDE ranging from non- to tri-BDEs. Thus, while the experimental conditions of the activated sludge studies are environmentally relevant, it is possible that the rates of degradation are too slow, or the cultures used are too specific, for the observed debromination to be significant in the environment. Overall, it appears that photodegradation may be more significant than biodegradation for deca-BDE sorbed to solids.

Transformation of deca-BDE in soil when plants are included in the test system

New evidence of significant transformation of deca-BDE in the environment when plants are introduced into the system has recently been presented on the basis of a greenhouse soil-plant study by Huang et al.[26] which provides extensive evidence that considerable transformation of deca-BDE to debrominated species ranging from nona- to hexa isomers, has occurred in the soil ranging from 6.5% to 34.6% with the average for the six plants being 20%. This transformation occurred in a period of 60 days. There is also substantial evidence of substances of concern in both shoots and roots. As some of these plants are eaten by animals/man then this is a route of direct exposure, and these results indicate considerable degradation of decabromodiphenyl ether to degradation products including substances of equivalent concern.

The REACH legislation does not specify what level of degradation of a parent molecule to substance(s) meeting the Article 57(a) to (e) criteria is to be regarded as meeting the substances of equivalent concern definition. However Article 56(6) specifies that where a preparation contains a SVHC levels below 0.1% are not subject to REACH authorisation procedures. By analogy, in the case of transformation products from the parent molecule it would appear that having a 0.1% transformation rate over a measurable time period would legally require the parent molecule to be regarded as meeting the Article 57(f) requirements. The relevant guidance document also appears to support this view.

In soil studies with six plant species, degradation (on average) of 20% of the starting deca-BDE has been observed over 60 days. The resulting levels of substances of concern ranged from 1.6-3.2% in the case of octa-isomers (only 2 of the 12 isomers were investigated) and 0.1-1.8% in the case of the hexa-isomers (only 4 of the 42 isomers were investigated).

This realistic study, which is of high relevance to what may happen in plant-soil systems in the environment, clearly classifies deca-BDE as a substance meeting Article 57(f) criteria as a substance of equivalent concern due to its degradation/transformation products.

Canadian Mesocosm studies

In an ongoing study by Orihel et al. (http://www.ontarioaquaculture.com/files/ELARES2008.pdf) the debromination rates of deca-BDE under natural field conditions has been investigated for the first time. This study provides substantial evidence of transformation of deca-BDE to its breakdown products in surface sediments in as early as 1 month. The main metabolite detected was nona-BDEs but smaller

amounts of octa-BDE were also detected at 1 and 8 months. Tri/tetra and penta-BDEs were also observed in the medium and high mesocosms, but near to the level of detection.

ENVIRONMENTAL MONITORING

Based on its chemical properties, deca-BDE is expected to be associated with either soils or sediment depending on whether it is released to soil or aquatic environment and, within these bulk compartments, deca-BDE is associated almost entirely with the solid phase. Thus, in the environment, it is expected that deca-BDE will be found primarily bound to solids in the water column, and bound to particles in the atmosphere.

Laboratory-based studies on the transformation of deca-BDE provide support for a conclusion that transformation to lower BDEs should be occurring in the environment, although the rate at which this is occurring and how this compares to rates of input are unknown. In the UK 10 year monitoring programme the congener BDE-126 was identified as a possible marker for deca-BDE transformation in the environment. BDE-126 has been detected in sediment and sewage sludge samples from 2007 (<1 g/kg dry weight) providing some evidence that this metabolite of deca-BDE and presumably other intermediate PBDE congeners are being formed in the environment. In addition, another possible degradation product BDE-202 used as marker for deca-BDE has also been found in abiotic and biotic matrices in the environment. Thus, some evidence are now accumulating that debromination of deca-BDE is occurring in the environment. There do not appear to be long-term time trend data on the concentrations of marker debromination congeners in environmental samples. However, it is unknown whether it would be expected that BDE-126 or BDE-202 would increase over time in environmental matrices as this would depend upon the environmental rate of debromination of deca-BDE relative to the degradation/metabolism of BDE-126 and BDE-202 themselves.

The available data show that deca-BDE occurs widely in indoor and outdoor dust and air. Such dust may therefore be considered a significant, uncontrollable and long-term diffuse source of deca-BDE in the environment. Although it is difficult to determine the extent of exposure of such dust to light, such exposure is likely to occur, especially in the atmosphere. It is therefore considered that deca-BDE on indoor dust can be a source of hepta-BDE congeners in the wider environment.

EVIDENCE OF DEBROMINATION IN VIVO

Mammals

Biotransformation of deca-BDE leading to the production of debrominated metabolites has been demonstrated to a limited extent in rodents. However the properties of the products thus generated are poorly understood. The following conclusions can be made on deca-BDE metabolism in mammals based on currently available studies mainly on laboratory rats:

- Reductive debromination to nona-, octa- and hepta-BDE is the likely first step in the metabolism of deca-BDE.
- Similar to fish, debromination may be the result of action by deiodinase enzymes.
- The debrominated neutral metabolites then appear to undergo hydroxylation to form phenols or catechols, potentially via an arene oxide. This could involve the action of cytochrome P450 enzymes.

- The hydroxylated PBDEs are likely to compete with thyroxiine for binding to TTR, a thyroxine transport protein present in blood serum
- The catechols are then methylated, potentially by the action of catechol-O-methyltransferase, to form the observed guiacols
- The guiacol metabolites could further oxidise to quinones, which are highly reactive and would bind to cellular macromolecules, possibly causing toxic effects.
- The reactive intermediates would also be subject to rapid conjugation via Phase II metabolic processes, leading to water-soluble metabolites which would be excreted via bile and faeces.

Birds

Only limited data are available concerning the potential for metabolic debromination of deca-BDE in birds. A study in which European starlings were exposed to deca-BDE over 76 days by means of implant, and a study examining the homologues patterns of hepta, octa and nona-BDEs in peregrine falcon eggs provides restricted evidence of biotransformation of deca-BDE leading to the production of at least octa- and nona-BDEs[35,47].

Fish

There is an abundance of evidence to support debromination of deca-BDE in at least some fish species (rainbow trout, lake trout, almost all cyprinid fish e.g. carp, minnows, zebra fish etc, American eels, sculpins). Deca-BDE is debrominated in fish as a first step in metabolism, producing at least debrominated hepta- to nona-BDEs but also potentially penta- and hexa-BDEs. Deiodinase enzymes which normally remove iodine from thyroxine appear to be likely candidates to catalyse this debromination pathway. Bromine has been observed to be preferentially removed from the *meta* and *para* positions.

Invertebrates

There appear to be no data suggesting that invertebrates in aquatic systems can debrominate deca-BDE. It is unlikely that invertebrates are able to debrominate deca-BDE given that invertebrates typically have less developed biotransforming enzymes than vertebrates.

REMAINING ISSUES

DEBROMINATION OF DECA-BDE

As of 2003 deca-BDE was considered to be "extensively metabolised, rapidly excreted and marginally distributed to adipose tissue"[38]. This was, until recently, taken to be reassuring in terms of human health risk; however, if deca-BDE metabolites (particularly debrominated derivatives) are excreted this could have environmental consequences. For example, the fact that deca-BDE and its metabolites are excreted efficiently in the faeces means that sewage is a potential source of environmental exposure, especially in areas where large volumes of human or animal sewage are released (e.g. around sewage works, areas of agricultural run-off). However, the fact that deca-BDE appears to undergo secondary oxidative metabolism leading to the formation of potentially reactive intermediates may allow metabolites to be "mopped up" (e.g. by binding to proteins in the gut), thus limiting the excretion of debrominated derivatives and their release into the environment.

Accordingly, the following questions about deca-BDE and its metabolites remain unanswered:

- What are the rates of formation of penta, hexa, hepta and octa-BDE from deca-BDE during vertebrate metabolism and in the environment?
- How are the debromination products of deca-BDE distributed in the environment?
- What is the timescale of removal or accumulation of these products in the environment?
- What quantities of these compounds are present in the environment as a consequence of the debromination of deca-BDE (bearing in mind that they may also arise from various other sources)?
- What is the biological significance of this process, bearing in mind that:
    - penta-BDE and hexa-BDEs are classified as vPvB substances
    - hepta-BDE has been identified as a Persistent Organic Pollutant (POP)
    - octa-BDE appears to be a reproductive toxin

OTHER METABOLITES OF DECA-BDE

There is evidence that oxidative metabolites of deca-BDE are formed in mammalian liver. No information is available concerning the toxicological properties of these metabolites, but they are an *a priori* cause for concern because oxidative metabolism is often a metabolic activation step leading to the production of reactive intermediates which may bind to proteins and/or DNA and thus induce a toxic response.

FURTHER QUESTIONS AND DATA GAPS

One of the greatest sources of uncertainty regarding the movement and fate of deca-BDE in abiotic media appears to be the extent of degradation in these environments. Although from the studies reviewed it is often suggested that environmental degradation is slow or negligible, photochemical and biological degradation of deca-BDE has been demonstrated in water, soil, sediments and house dust under laboratory conditions. Deca-BDE has also been shown to be susceptible to abiotic degradation by metal oxides that occur naturally in soils and sediments. Yet, the significance of these pathways during storage or transport of deca-BDE in the environment is unknown. To date, few studies have addressed deca-BDE degradation experimentally (with the exception of photodegradation) studies and strong evidence for degradation *in situ* appears to be lacking.

The extent to which deca-BDE is bioavailable in different environmental compartments may determine the amount of microbial degradation that occurs, but no direct studies of deca-BDE bioavailability appear to have been published. Bioavailability in soil and sediments is often a function of sorption to mineral particles or organic matter and there is evidence showing limited deca-BDE bioavailability and biodegradation in soil artificially amended with deca-BDE. Thus, bioavailability and bioaccumulation may be limited by strong sorption to mineral or organic constituents in soils and sediments and aqueous microbial transformations, yet very few studies have addressed these issues. Experiments investigating deca-BDE bioavailability and degradation are needed. Studies tracking the fate of artificial doses of deca-BDE in biotic and abiotic media, including the use of isotopically labeled deca-BDE, would be useful.

Outside the laboratory, few studies seem to have addressed the consequences of deca-BDE uptake and accumulation by organisms for their fitness although the consequences of PBDE content for reproductive behavior and immunosuppression appear to have been observed.

It seems to be very difficult to determine which fish species can and cannot debrominate deca-BDE. Until the enzyme system catalysing the debromination is identified, it is almost impossible to tell which species can and cannot debrominate deca-BDE. Additionally, the possibility of metabolic formation of MeO-BDE and HO-BDE from deca-BDE requires further investigation.

It is also very difficult to determine the rate at which deca-BDE will debrominate in the environment. The rates may be low under standard conditions and are also difficult to assess because debromination can occur via different routes (photolysis, redox reactions, bacteria).

OVERALL CONCLUSIONS

The ACHS has reviewed recent publications in the literature on deca-BDE, taking into account international opinion. There is no new evidence to alter the current regulatory status of deca-BDE itself, which whilst being very persistent does not meet the bioconcentration criteria for authorisation and is not classified as dangerous.

Paradoxically, the evidence for environmental degradation provides reassurance that the compound is not persistent, while simultaneously raising concerns about its potential to give rise to SVHCs as a result of transformation. It would appear that deca-BDE lies on the borderline of the "very persistent" classification. For example, data from the study of Huang et al.[26] Indicate significant breakdown of deca-BDE (some 20% in 60 days, ranging from 6.5% to 34.6%), yet the resulting half-life in soil is >180 days (actually 186 days for the 20% case), thus just meeting the criterion for a very persistent compound.

With regard to the significance of breakdown products it is necessary to show that deca-BDE can degrade or be transformed in the environment to at least the octa-, hepta- or to hexa-BDE isomers in order to trigger potential action under Article 57(f) of REACH. Furthermore, in order to meet the Article 57(f) definitions such degradation has to occur at more than trivial quantities. New literature studies demonstrate the potential for debromination to occur by photochemistry, microbial degradation, metabolism in fish, as well as transformation in one soil/plant study under controlled conditions, plus evidence of degradation in sediments from a mesocosm study.

However, demonstrating that this degradation occurs in the real environment is more difficult due to the previous use of octa-BDE as well as penta-BDE, which will also potentially degrade in the environment. Additionally many studies only measure a few of the potential isomers and often do not state that there is the potential for 12 octabrominated isomers, 24 heptabrominated isomers and 42 hexabrominated isomers to arise from deca-BDE, as well as from the previous used octa-BDE.

There is still a need for clean environmental studies in which only deca-BDE is present at the start of the experiment. A 2010 plant/soil study under controlled conditions clearly demonstrated degradation as well as transport through air of the parent compound, which explains the widespread appearance of these brominated compounds in the environment. However, this was run at 20/25° C using unspecified lamps giving a specified light intensity. This work requires repetition in an outside environment to confirm the findings. The ACHS recommends that such a study should be progressed. With regard to sediments, a Canadian mesocosm study which is currently in progress will hopefully provide answers.

These brominated compounds accumulate in soils and sediments and relevant studies in these two environmental compartments would be of value in supporting a decision as to whether deca-BDE meets the Article 57(f) criteria for a substance of equivalent concern. The major use of deca-BDE is now in textiles so it would also seem sensible to devise an experiment to determine whether dusts derived from this use and containing only deca-BDE do show photodegradation in a realistic setting.

The ACHS is concerned that its conclusions regarding the degradation of deca-BDE by soil and plants are, of necessity, based upon a single study[26]. Circumstantial evidence indicates that there is potential for decaBDE to debrominate to, for example, hexa- and heptaBDE in the environment, at levels that are of concern. The ACHS recognises that independent verification of the soil/plant study, plus a possible dust study considering photodegradation, may be prudent and desirable. Completion of the Canadian mesocosm study should provide the required quantitative data on sediments. However, the ACHS does not think that additional work should be a reason to delay starting the decision-making process.

ACHS ADVICE TO REGULATORS

The ACHS concludes that there is strong, but incomplete, scientific evidence indicating that deca-BDE has the potential to undergo transformation to lower brominated congeners in the environment. The additional data reported since 2007 have added to the concerns expressed previously by the committee. In particular, there is an ever-increasing body of evidence indicating that deca-BDE may be degraded to lower congeners that are SVHCs.

Deca-BDE itself does not meet the current criteria for classification as an SVHC. However, the ACHS is satisfied that there is conclusive qualitative evidence that this compound can undergo degradation to lower brominated congeners. Estimates vary as to the rate and extent to which this degradation is likely to occur in the environment. The committee recognises the difficulty of obtaining quantitative evidence given the physical properties of deca-BDE itself (including low aqueous solubility), the large number of potential breakdown products and the fact that these products can be produced from other parent materials as well as from deca-BDE.

The existence of strong qualitative evidence, together with some quantification in experimental systems, has convinced the ACHS that deca-BDE has the potential to undergo environmental degradation to SVHCs. The remaining question is: To what extent can qualitative evidence be relied upon in the regulatory context? If qualitative evidence is considered sufficient for regulatory purposes, then the ACHS considers that deca-BDE meets the Article 57(f) criteria for classification as a Substance of Equivalent Concern. In this case, the committee recommends timely preparation of a Risk Management Options paper and Annex XV dossier.

APPENDIX 1: CONGENER NUMBERS AND NAMES

BDE-1	2-Bromodiphenyl ether
BDE-2	3-Bromodiphenyl ether
BDE-3	4-Bromodiphenyl ether
BDE-4	2, 2'-Dibromodiphenyl ether
BDE-5	2, 3-Dibromodiphenyl ether
BDE-6	2, 3'-Dibromodiphenyl ether
BDE-7	2, 4-Dibromodiphenyl ether
BDE-8	2, 4'-Dibromodiphenyl ether
BDE-9	2, 5-Dibromodiphenyl ether
BDE-10	2, 6-Dibromodiphenyl ether
BDE-11	3, 3'-Dibromodiphenyl ether
BDE-12	3, 4-Dibromodiphenyl ether
BDE-13	3, 4'-Dibromodiphenyl ether
BDE-14	3, 5-Dibromodiphenyl ether
BDE-15	4, 4'-Dibromodiphenyl ether
BDE-16	2, 2', 3-Tribromodiphenyl ether
BDE-17	2, 2', 4-Tribromodiphenyl ether
BDE-18	2, 2', 5-Tribromodiphenyl ether
BDE-19	2, 2', 6-Tribromodiphenyl ether
BDE-20	2, 3, 3'-Tribromodiphenyl ether
BDE-21	2, 3, 4-Tribromodiphenyl ether
BDE-22	2, 3, 4'-Tribromodiphenyl ether
BDE-23	2, 3, 5-Tribromodiphenyl ether
BDE-24	2, 3, 6-Tribromodiphenyl ether
BDE-25	2, 3', 4-Tribromodiphenyl ether
BDE-26	2, 3', 5-Tribromodiphenyl ether
BDE-27	2, 3', 6-Tribromodiphenyl ether
BDE-28	2, 4, 4'-Tribromodiphenyl ether
BDE-29	2, 4, 5-Tribromodiphenyl ether
BDE-30	2, 4, 6-Tribromodiphenyl ether
BDE-31	2, 4', 5-Tribromodiphenyl ether
BDE-32	2, 4', 6-Tribromodiphenyl ether
BDE-33	2, 3', 4'-Tribromodiphenyl ether
BDE-34	2, 3', 5'-Tribromodiphenyl ether
BDE-35	3, 3', 4-Tribromodiphenyl ether
BDE-36	3, 3', 5-Tribromodiphenyl ether
BDE-37	3, 4, 4'-Tribromodiphenyl ether
BDE-38	3, 4, 5-Tricholodiphenyl ether
BDE-39	3, 4', 5-Tribromodiphenyl ether
BDE-40	2, 2', 3, 3'-Tetrabromodiphenyl ether
BDE-41	2, 2', 3, 4-Tetrabromodiphenyl ether
BDE-42	2, 2', 3, 4'-Tetrabromodiphenyl ether
BDE-43	2, 2', 3, 5-Tetrabromodiphenyl ether

BDE-44	2, 2', 3, 5'-Tetrabromodiphenyl ether
BDE-45	2, 2', 3, 6-Tetrabromodiphenyl ether
BDE-46	2, 2', 3, 6'-Tetrabromodiphenyl ether
BDE-47	2, 2', 4, 4'-Tetrabromodiphenyl ether
BDE-48	2, 2', 4, 5-Tetrabromodiphenyl ether
BDE-49	2, 2', 4, 5'-Tetrabromodiphenyl ether
BDE-50	2, 2', 4, 6-Tetrabromodiphenyl ether
BDE-51	2, 2', 4, 6'-Tetrabromodiphenyl ether
BDE-52	2, 2', 5, 5'-Tetrabromodiphenyl ether
BDE-53	2, 2', 5, 6'-Tetrabromodiphenyl ether
BDE-54	2, 2', 6, 6'-Tetrabromodiphenyl ether
BDE-55	2, 3, 3', 4-Tetrabromodiphenyl ether
BDE-56	2, 3, 3', 4'-Tetrabromodiphenyl ether
BDE-57	2, 3, 3', 5-Tetrabromodiphenyl ether
BDE-58	2, 3, 3', 5'-Tetrabromodiphenyl ether
BDE-59	2, 3, 3', 6-Tetrabromodiphenyl ether
BDE-60	2, 3, 4, 4'-Tetrabromodiphenyl ether
BDE-61	2, 3, 4, 5-Tetrabromodiphenyl ether
BDE-62	2, 3, 4, 6-Tetrabromodiphenyl ether
BDE-63	2, 3, 4', 5-Tetrabromodiphenyl ether
BDE-64	2, 3, 4', 6-Tetrabromodiphenyl ether
BDE-65	2, 3, 5, 6-Tetrabromodiphenyl ether
BDE-66	2, 3', 4, 4'-Tetrabromodiphenyl ether
BDE-67	2, 3', 4, 5-Tetrabromodiphenyl ether
BDE-68	2, 3', 4, 5'-Tetrabromodiphenyl ether
BDE-69	2, 3', 4, 6-Tetrabromodiphenyl ether
BDE-70	2, 3', 4', 5-Tetrabromodiphenyl ether
BDE-71	2, 3', 4', 6-Tetrabromodiphenyl ether
BDE-72	2, 3', 5, 5'-Tetrabromodiphenyl ether
BDE-73	2, 3', 5', 6-Tetrabromodiphenyl ether
BDE-74	2, 4, 4', 5-Tetrabromodiphenyl ether
BDE-75	2, 4, 4', 6-Tetrabromodiphenyl ether
BDE-76	2, 3', 4', 5'-Tetrabromodiphenyl ether
BDE-77	3, 3', 4, 4'-Tetrabromodiphenyl ether
BDE-78	3, 3', 4, 5-Tetrabromodiphenyl ether
BDE-79	3, 3', 4, 5'-Tetrabromodiphenyl ether
BDE-80	3, 3', 5, 5'-Tetrabromodiphenyl ether
BDE-81	3, 4, 4', 5-Tetrabromodiphenyl ether
BDE-82	2, 2', 3, 3', 4-Pentabromodiphenyl ether
BDE-83	2, 2', 3, 3', 5-Pentabromodiphenyl ether
BDE-84	2, 2', 3, 3', 6-Pentabromodiphenyl ether
BDE-85	2, 2', 3, 4, 4'-Pentabromodiphenyl ether
BDE-86	2, 2', 3, 4, 5-Pentabromodiphenyl ether
BDE-87	2, 2', 3, 4, 5'-Pentabromodiphenyl ether
BDE-88	2, 2', 3, 4, 6-Pentabromodiphenyl ether
BDE-89	2, 2', 3, 4, 6'-Pentabromodiphenyl ether

BDE-90	2, 2', 3, 4', 5-Pentabromodiphenyl ether
BDE-91	2, 2', 3, 4', 6-Pentabromodiphenyl ether
BDE-92	2, 2', 3, 5, 5'-Pentabromodiphenyl ether
BDE-93	2, 2', 3, 5, 6-Pentabromodiphenyl ether
BDE-94	2, 2', 3, 5, 6'-Pentabromodiphenyl ether
BDE-95	2, 2', 3, 5', 6-Pentabromodiphenyl ether
BDE-96	2, 2', 3, 6, 6'-Pentabromodiphenyl ether
BDE-97	2, 2', 3, 4', 5'-Pentabromodiphenyl ether
BDE-98	2, 2', 3, 4', 6'-Pentabromodiphenyl ether
BDE-99	2, 2', 4, 4', 5-Pentabromodiphenyl ether
BDE-100	2, 2', 4, 4', 6-Pentabromodiphenyl ether
BDE-101	2, 2', 4, 5, 5'-Pentabromodiphenyl ether
BDE-102	2, 2', 4, 5, 6'-Pentabromodiphenyl ether
BDE-103	2, 2', 4, 5', 6-Pentabromodiphenyl ether
BDE-104	2, 2', 4, 6, 6'-Pentabromodiphenyl ether
BDE-105	2, 3, 3', 4, 4'-Pentabromodiphenyl ether
BDE-106	2, 3, 3', 4, 5-Pentabromodiphenyl ether
BDE-107	2, 3, 3', 4', 5-Pentabromodiphenyl ether
BDE-108	2, 3, 3', 4, 5'-Pentabromodiphenyl ether
BDE-109	2, 3, 3', 4, 6-Pentabromodiphenyl ether
BDE-110	2, 3, 3', 4', 6-Pentabromodiphenyl ether
BDE-111	2, 3, 3', 5, 5'-Pentabromodiphenyl ether
BDE-112	2, 3, 3', 5, 6-Pentabromodiphenyl ether
BDE-113	2, 3, 3', 5', 6-Pentabromodiphenyl ether
BDE-114	2, 3, 4, 4', 5-Pentabromodiphenyl ether
BDE-115	2, 3, 4, 4', 6-Pentabromodiphenyl ether
BDE-116	2, 3, 4, 5, 6-Pentabromodiphenyl ether
BDE-117	2, 3, 4', 5, 6-Pentabromodiphenyl ether
BDE-118	2, 3', 4, 4', 5-Pentabromodiphenyl ether
BDE-119	2, 3', 4, 4', 6-Pentabromodiphenyl ether
BDE-120	2, 3', 4, 5, 5'-Pentabromodiphenyl ether
BDE-121	2, 3', 4, 5', 6-Pentabromodiphenyl ether
BDE-122	2, 3, 3', 4', 5'-Pentabromodiphenyl ether
BDE-123	2, 3', 4, 4', 5'-Pentabromodiphenyl ether
BDE-124	2, 3', 4', 5, 5'-Pentabromodiphenyl ether
BDE-125	2, 3', 4', 5', 6-Pentabromodiphenyl ether
BDE-126	3, 3', 4, 4', 5-Pentabromodiphenyl ether
BDE-127	3, 3', 4, 5, 5'-Pentabromodiphenyl ether
BDE-128	2, 2', 3, 3', 4, 4'-Hexabromodiphenyl ether
BDE-129	2, 2', 3, 3', 4, 5-Hexabromodiphenyl ether
BDE-130	2, 2', 3, 3', 4, 5'-Hexabromodiphenyl ether
BDE-131	2, 2', 3, 3', 4, 6-Hexabromodiphenyl ether
BDE-132	2, 2', 3, 3', 4, 6'-Hexabromodiphenyl ether
BDE-133	2, 2', 3, 3', 5, 5'-Hexabromodiphenyl ether
BDE-134	2, 2', 3, 3', 5, 6-Hexabromodiphenyl ether
BDE-135	2, 2', 3, 3', 5, 6'-Hexabromodiphenyl ether

BDE-136	2, 2', 3, 3', 6, 6'-Hexabromodiphenyl ether
BDE-137	2, 2', 3, 4, 4', 5-Hexabromodiphenyl ether
BDE-138	2, 2', 3, 4, 4', 5'-Hexabromodiphenyl ether
BDE-139	2, 2', 3, 4, 4', 6-Hexabromodiphenyl ether
BDE-140	2, 2', 3, 4, 4', 6'-Hexabromodiphenyl ether
BDE-141	2, 2', 3, 4, 5, 5'-Hexabromodiphenyl ether
BDE-142	2, 2', 3, 4, 5, 6-Hexabromodiphenyl ether
BDE-143	2, 2', 3, 4, 5, 6'-Hexabromodiphenyl ether
BDE-144	2, 2', 3, 4, 5', 6-Hexabromodiphenyl ether
BDE-145	2, 2', 3, 4, 6, 6'-Hexabromodiphenyl ether
BDE-146	2, 2', 3, 4', 5, 5'-Hexabromodiphenyl ether
BDE-147	2, 2', 3, 4', 5, 6-Hexabromodiphenyl ether
BDE-148	2, 2', 3, 4', 5, 6'-Hexabromodiphenyl ether
BDE-149	2, 2', 3, 4', 5', 6-Hexabromodiphenyl ether
BDE-150	2, 2', 3, 4', 6, 6'-Hexabromodiphenyl ether
BDE-151	2, 2', 3, 5, 5', 6-Hexabromodiphenyl ether
BDE-152	2, 2', 3, 5, 6, 6'-Hexabromodiphenyl ether
BDE-153	2, 2', 4, 4', 5, 5'-Hexabromodiphenyl ether
BDE-154	2, 2', 4, 4', 5, 6'-Hexabromodiphenyl ether
BDE-155	2, 2', 4, 4', 6, 6'-Hexabromodiphenyl ether
BDE-156	2, 3, 3', 4, 4', 5-Hexabromodiphenyl ether
BDE-157	2, 3, 3', 4, 4', 5'-Hexabromodiphenyl ether
BDE-158	2, 3, 3', 4, 4', 6-Hexabromodiphenyl ether
BDE-159	2, 3, 3', 4, 5, 5'-Hexabromodiphenyl ether
BDE-160	2, 3, 3', 4, 5, 6-Hexabromodiphenyl ether
BDE-161	2, 3, 3', 4, 5', 6-Hexabromodiphenyl ether
BDE-162	2, 3, 3', 4', 5, 5'-Hexabromodiphenyl ether
BDE-163	2, 3, 3', 4', 5, 6-Hexabromodiphenyl ether
BDE-164	2, 3, 3', 4', 5', 6-Hexabromodiphenyl ether
BDE-165	2, 3, 3', 5, 5', 6-Hexabromodiphenyl ether
BDE-166	2, 3, 4, 4', 5, 6-Hexabromodiphenyl ether
BDE-167	2, 3', 4, 4', 5, 5'-Hexabromodiphenyl ether
BDE-168	2, 3', 4, 4', 5', 6-Hexabromodiphenyl ether
BDE-169	3, 3', 4, 4', 5, 5'-Hexabromodiphenyl ether
BDE-170	2, 2', 3, 3', 4, 4', 5-Heptabromodiphenyl ether
BDE-171	2, 2', 3, 3', 4, 4', 6-Heptabromodiphenyl ether
BDE-172	2, 2', 3, 3', 4, 5, 5'-Heptabromodiphenyl ether
BDE-173	2, 2', 3, 3', 4, 5, 6-Heptabromodiphenyl ether
BDE-174	2, 2', 3, 3', 4, 5, 6'-Heptabromodiphenyl ether
BDE-175	2, 2', 3, 3', 4, 5', 6-Heptabromodiphenyl ether
BDE-176	2, 2', 3, 3', 4, 6, 6'-Heptabromodiphenyl ether
BDE-177	2, 2', 3, 3', 4, 5', 6'-Heptabromodiphenyl ether
BDE-178	2, 2', 3, 3', 5, 5', 6-Heptabromodiphenyl ether
BDE-179	2, 2', 3, 3', 5, 6, 6'-Heptabromodiphenyl ether
BDE-180	2, 2', 3, 4, 4', 5, 5'-Heptabromodiphenyl ether
BDE-181	2, 2', 3, 4, 4', 5, 6-Heptabromodiphenyl ether

BDE-182	2, 2', 3, 4, 4', 5, 6'-Heptabromodiphenyl ether
BDE-183	2, 2', 3, 4, 4', 5', 6-Heptabromodiphenyl ether
BDE-184	2, 2', 3, 4, 4', 6, 6'-Heptabromodiphenyl ether
BDE-185	2, 2', 3, 4, 5, 5', 6-Heptabromodiphenyl ether
BDE-186	2, 2', 3, 4, 5, 6, 6'-Heptabromodiphenyl ether
BDE-187	2, 2', 3, 4', 5, 5', 6-Heptabromodiphenyl ether
BDE-188	2, 2', 3, 4', 5, 6, 6'-Heptabromodiphenyl ether
BDE-189	2, 3, 3', 4, 4', 5, 5'-Heptabromodiphenyl ether
BDE-190	2, 3, 3', 4, 4', 5, 6-Heptabromodiphenyl ether
BDE-191	2, 3, 3', 4, 4', 5', 6-Heptabromodiphenyl ether
BDE-192	2, 3, 3', 4, 5, 5', 6-Heptabromodiphenyl ether
BDE-193	2, 3, 3', 4', 5, 5', 6-Heptabromodiphenyl ether
BDE-194	2, 2', 3, 3', 4, 4', 5, 5'-Octabromodiphenyl ether
BDE-195	2, 2', 3, 3', 4, 4', 5, 6-Octabromodiphenyl ether
BDE-196	2, 2', 3, 3', 4, 4', 5, 6'-Octabromodiphenyl ether
BDE-197	2, 2', 3, 3', 4, 4', 6, 6'-Octabromodiphenyl ether
BDE-198	2, 2', 3, 3', 4, 5, 5', 6-Octabromodiphenyl ether
BDE-199	2, 2', 3, 3', 4, 5, 5', 6'-Octabromodiphenyl ether
BDE-200	2, 2', 3, 3', 4, 5, 6, 6'-Octabromodiphenyl ether
BDE-201	2, 2', 3, 3', 4, 5', 6, 6'-Octabromodiphenyl ether
BDE-202	2, 2', 3, 3', 5, 5', 6, 6'-Octabromodiphenyl ether
BDE-203	2, 2', 3, 4, 4', 5, 5', 6-Octabromodiphenyl ether
BDE-204	2, 2', 3, 4, 4', 5, 6, 6'-Octabromodiphenyl ether
BDE-205	2, 3, 3', 4, 4', 5, 5', 6-Octabromodiphenyl ether
BDE-206	2, 2', 3, 3', 4, 4', 5, 5', 6-Nonabromodiphenyl ether
BDE-207	2, 2', 3, 3', 4, 4', 5, 6, 6'-Nonabromodiphenyl ether
BDE-208	2, 2', 3, 3', 4, 5, 5', 6, 6'-Nonabromodiphenyl ether

BDE-209	DECABROMODIPHENYL ETHER

APPENDIX 2: BIOACCUMULATION AND BIOMAGNIFICATION

The existing evidence for bioaccumulation of deca-BDE does not support a conclusion of bioaccumulation (draft addendum environmental risk assessment report for deca-BDE). It is also thought that given the variability in the data and lack of consistent evidence for significant bioaccumulation or biomagnification of deca-BDE, a decision cannot be made about whether deca-BDE should be considered to meet either the B or vB criteria. However, the key issue is whether the degradation products (lower congeners) meet the PBT or vPvB criteria, which if they do, would mean that deca-BDE does meet the definition of a substance of equivalent concern.

The more brominated PBDE congeners have higher octanol-water partition coefficient (K_{ow}) and therefore are more hydrophobic. Hydrophobicity is an important predictor of bioaccumulation and biomagnification. However, molecular size could be another factor affecting bioaccumulation and biomagnifications, for example absorption of more brominated congeners (e.g. deca-BDE) may be hindered in biota due to the larger molecular size (>9.5 Å)[58].

Nevertheless, a few studies have been published indicating that aquatic organisms are able to accumulate deca-BDE [36,53,59-64], although the level of uptake is likely to be low. The mean levels of deca-BDE concentrations have ranged from sub-ppb to ppm levels (21.6 µg/g-lipid in crayfish, *Cambarus puneticambarus*).

Bioaccumulation and biomagnifications of PBDE has been investigated in a food web from Lake Michigan in a new study by Kuo et al.[58] which has been reviewed. The food web analysed consisted of quantified bioaccumulation in plankton, *Diporeira* spp, Chinook salmon (*Oncorhynchus tshawytscha*), lake trout (*Salvelinus namaycush*) and lake whitefish (*Coregonus clupeaformis*). The seven PBDE congeners analysed included BDE-47 (tetra-BDE), BDE-99 and BDE-100 (penta-BDE), BDE-153 and BDE-154 (hexa-BDE), BDE-205 (octa-BDE) and BDE-209 (deca-BDE). The main conclusions on bioaccumulation and biomagnifications from this study included:

- There were no statistically significant differences between liver and muscle PBDE concentrations within each fish species. This is in accordance to other reports [65] and suggests a uniform distribution of BDE-47, 99, 100, 153, 154 between liver and muscle in the fish studied.
- Across all fish tissues and invertebrates, individual PBDE concentrations of BDE-47, 99, 100, 153 and 154 (on a wet weight basis) were significantly and positively related to lipid content except for deca-BDE, for which no significant trends were observed. The reason behind this lack of relationship with lipid content is likely due to the fact that deca-BDE has a higher affinity with serum proteins instead of lipids [38]. In contrast, the positive linear trends observed for BDE-47, 99, 100, 153 and 154 suggest bioaccumulation of PBDEs is correlated with lipid content since higher lipid content increased the capacity for PBDE accumulation.
- The biomagnification factor (BMF) calculation and the isotope analysis of the PBDE concentrations indicated that BDE-47 and 100 biomagnified. However, deca-BDE concentrations decreased at higher tropic levels - a significant negative correlation between deca-BDE and tropic level was found in this food web - suggesting that deca -BDE is not

taken up by organisms at higher positions in the food chain, but instead a partial uptake and/or biotransformation of deca-BDE while tropic transfer within members of the food web of Lake Michigan was suggested.

- The highest levels of deca-BDE (140 µg/g-lipid) was found in Diporeia, a benthic organism. Higher concentrations of deca-BDE has been measured for sediment from Lake Michigan concentrations of deca-BDE at least one order higher than the sum of the other 9 common congeners [66]. Thus, sediment is a likely source of deca-BDE to Diporeia.

These results are in agreement with those of Burreau et al.[67] from three different fish species in the Baltic Sea i.e. pike (*Esox lucius*), perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*). The values for BDE-47 and 100 are also similar to findings from several fish species in Lake Winnipeg in Canada (i.e. walleye, whitefish, emerald shiner, goldeye, white sucker, turbot [63]), although these authors did not observe significant biomagnification for the two congeners BDE-47 and 100. In contrast to Kuo et al.[53], Law et al.[63]reported significant biomagnifications for deca-BDE.

The results of Kuo et al.[53] are in agreement with another study by Wang et al., [68] who studied the bioaccumulation of deca-BDE in organisms downstream of a waste water treatment plant of Gaobeidian Lake, Beijing, China. Deca-BDE was not detectable in either the effluent samples or the lake water samples but was present in the sediment cores at a mean concentration of 237 µg/kg dry weight. For the aquatic species, the highest concentrations of deca-BDE were found in spirogyra, March brown, coccid and zooplankton with lower levels being found in the fish and turtles. Based on nitrogen isotope ratios it was determined that java tilapia was at the highest tropic level and no indication for biomagnifications was found in the available data. As in Kuo et al.[53], the study also determined the levels of lower brominated PBDE congeners (tri- to hepta-BDEs) and found a linear relationship between bioaccumulation factor in fish and the number of bromine atoms in the molecule; hepta-BDE (6900), octa-BDE (2900), nona-BDE (1200) and deca-BDE (500) suggesting that the lower congeners bioaccumulate whereas the higher brominated congeners (i.e. deca-BDE) does not.

This evidence indicates that the lower brominated congeners (BDE-47 and 100) bioaccumulate and biomagnify in fish, whereas deca-BDE does not.

It should be noted that biomagnifications and biotransformation of deca-BDE is related to specific metabolism and dietary habit of each species. This can explain why BMF of a PBDE congener differ in different tropic interactions. It is also clear that sediments are a likely source of deca-BDE to benthic organisms living in close proximity to sediment. It is also known that deca-BDE degrades much slower when adsorbed to sediment and soils, benthic organisms could therefore be one of the main dietary sources of deca-BDE for fish especially in estuaries and rivers. Also, stationary, bottom-living fish (such as the flounder etc.) often burrowing into and ingesting sediment while feeding could potentially be exposed to significant deca-BDE levels in the estuaries.

APPENDIX 3: PBT/VPVB PROPERTIES OF LOWER PBDE CONGENERS WITHIN THE MEANING OF REACH ANNEX XIII

As only limited amounts of data are available on the properties of individual PBDE congener groups a read-across approach[7] has been used to conclude on their properties. The original data are summarised in the ESR risk assessment reports for commercial octabromodiphenyl ether[8] and pentabromodiphenyl ether[9] unless otherwise stated.

Persistence: Hexa-, hepta-, octa- and nona-BDEs can all be considered to be very persistent (vP), based on read across from sediment studies with deca-BDE (see main text) and the finding that 2,2'4,4'-tetrabromodiphenyl ether did not degrade significantly in an anaerobic sediment study over 32 weeks in the dark at 22°C. However, as is the case with deca-BDE, slow degradation will occur (in the study with 2, 2'4, 4'-tetrabromodiphenyl ether, one to three peaks eluted before the parent compound after 32 weeks, one of which was significant. The identity of this degradant was not determined).

Bioaccumulation: The measured fish bioconcentration factor (BCF) for hexa-BDE is 5,640 l/kg. Therefore hexa-BDEs can be considered to be very bioaccumulative (vB) substances (i.e. BCF > 5,000 l/kg).

No measured bioconcentration data are available for hepta-BDEs. Estimates (based on a read-across approach) suggest that the fish BCF would be in the range 144-1,580 l/kg. This is below the bioaccumulative (B) criterion of 2,000 l/kg.

No significant accumulation of octa-BDE was observed in an 8-week fish bioconcentration study. Therefore it can be concluded that octa-BDEs do not meet the B or vB criteria. It would be expected that the bioconcentration potential of nona-BDEs would be between that of octa-BDEs and deca-BDE. Since deca-BDE and octa-BDEs are not considered to meet the B/vB criterion based on fish BCF, it can also be concluded that nona-BDEs do not meet the B/vB criteria either.

As is the case with deca-BDE, it is likely that fish BCFs may underestimate the actual potential for accumulation for these substances, and a bioaccumulation factor (BAF) may be more relevant. Based on the results of the study by Wang et al.(2007), BAFs of around 1,200, 2,900 and 6,900 l/kg can be estimated for nona-, octa- and hepta-BDE respectively (although it is not clear if these are lipid normalised or wet weight values).

Toxicity: No relevant toxicity data are available for any individual lower PBDE congener group. Tests using a commercial octabromodiphenyl ether product showed no effects in acute toxicity tests with fish or in longer-term studies using *Daphnia magna*. As the main components were hepta- and octa-BDEs (with smaller amounts of hexa-BDEs) it can be tentatively concluded that these components do not meet the T criterion based on the limited aquatic toxicity data currently available.

[7] See Appendix 1 of http://publications.environment-agency.gov.uk/pdf/SCHO0909BQYZ-e-e.pdf.
[8] http://ecb.jrc.ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/octareport014.pdf
[9] http://ecb.jrc.ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/penta_bdpereport015.pdf

Commercial octabromodiphenyl ether is classified as toxic to reproduction Category 1B (H360DF - May damage the unborn child. Suspected of damaging fertility) in Annex VI of Regulation (EC) No 1272/2008[10]. The composition of the test substance used in the test that leads to this developmental toxicity hazard classification was 0.2 per cent penta-BDE, 8.6 per cent hexa-BDE, 45 per cent hepta-BDE, 33.5 per cent octa-BDE, 11.2 per cent nona-BDE and 1.4 per cent deca-BDE[11]. This classification would be sufficient for the commercial octabromodiphenyl ether product to meet the Annex XIII criteria for toxicity, and it would also satisfy the SVHC criteria in accordance with Article 57c had it not been banned already. It is not known which components of the commercial product contribute to the toxicity that led to this classification but the two main components to which the animals would have been exposed would be the hepta-BDEs and octa-BDEs, and exposure to the hexa-BDEs could also have been significant (as these have a higher bioaccumulation potential than the higher brominated congeners). It can be assumed that this classification would also apply to the main components of the commercial product and so these components can be considered to meet the T criterion.

The lack of relevant data means that it is not possible to conclude on the mammalian toxicity of nona-BDE.

Summary

Hexa-BDE can be considered to meet the criteria for both a vPvB and a PBT substance. It has recently been added to the Stockholm Convention on persistent organic pollutants (POPs).

Hepta-BDE can be considered to be P/vP and T but does not appear, by read across, to meet the specific B/vB criteria based on fish BCF data (although this latter conclusion is uncertain - high BAFs are predicted). The substance has recently been confirmed as a POP under the Stockholm Convention. Therefore the substance can be considered to be equivalent to a PBT substance.

The status of octa- and nona-BDEs is less clear. Neither would be expected to meet the B/vB criteria based on the available data and so they are considered not to be PBT/vPvB substances. However, the human health hazard classification for commercial octabromodiphenyl ether products satisfies the SVHC criteria under Article 57c.

[10] 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).

[11] The composition of the test substance that led to the fertility classification is not reported but is expected to have been broadly similar.

APPENDIX 4: REPRODUCTIVE TOXICITY OF COMMERCIAL OCTA-BDE PRODUCTS

Product tested	Composition	Species/ strain	Dose and route	Observations
DE-79	10.5% Hexa 45.5% Hepta 37.9% Octa 13.1% Nona 1.3% Deca	Rat Charles River COBS CD	2.5, 10, 15, 25, 50 mg/kg/day p.o. on gestational days 6-15.	• Decreased maternal body weight on days 16-20. • Increased late resorptions and reduced mean fetal weight. • Suggestions of post-implantation loss of embryos (not statistically significant) • Sporadic fetal abnormalities associated with retarded ossification (anasarca, bent limb bones and unilateral absence of 13th rib) N.B. The reported composition of this material adds up to more than 100%
Saytex-111	8.6% Hexa 45% Hepta 33.5% Octa 11.2% Nona 1.4% Deca	Rat Strain?	2.5, 10, 25 mg/kg/day p.o. on gestational days 6-15; sacrificed on d20.	• Decreased average maternal body weight and body weight gain at 25 mg/kg/day. • No evidence of maternal toxicity. • No effects on corpora lutea or implantations. • High incidence of resorption and low average fetal bodyweight (2.10 g (25 mg/kg/day) vs. 3.38 g (control). • Significant fetal lethality, fetal malformations and delayed skeletal ossification at 25 mg/kg/day.
		Rabbit New Zealand White	2, 5, 15 mg/kg/day p.o. on gestational days 7-19; offspring examined on d28.	• No significant differences in: o Number of pregnancies o Number of litters with viable pups o Number of corpora lutea/dam o Number of implantations/dam o Number of live fetuses/litter o Proportion of fetuses resorbed o Decreased fetal body weight and delayed ossification at doses above 5 mg/kg/day.
FR-1208	8.2% Hexa 58.8% Hepta 25.3% Octa 6.7% Nona 0.9% Deca	Rat Sprague-Dawley CD	2.5, 10, 25 mg/kg/day p.o. on gestational days 6-15; sacrificed on d20.	• No adverse effects on the dam at any dose. • Some post-implantation loss at 10 and 25 mg/kg/day, but within historical control values for this species and strain. • No treatment-related skeletal malformations or delayed ossification at any dose. N.B. The test material used for this study contained a lower percentage of octa-BDE and a higher percentage of hepta-BDE than the materials used in the other two rat studies.

Summarised from http://ecb.jrc.ec.europa.eu/documents/Existing-Chemicals/RISK_ASSESSMENT/REPORT/octareport014.pdf.

APPENDIX 5: DEVELOPMENTAL NEUROTOXICITY OF POLYBROMINATED DIPHENYL ETHERS

Degree of bromination	Congener tested /purity	Species/ strain	Dose and route	Time of testing	Results	Comments/other findings/references
Penta-BDE	BDE-99 >99%	C57BL/6J mouse	0.4, 0.8, 4.0, 8.0, 16.0 mg/kg p.o. (single dose) on pnd10.	2,5 and 8 months after dosing	• No clinical signs of toxicity • Irreversible changes in spontaneous behavior in males and females • Dose and time related. • Worsened with age, • Occurred at all but lowest dose and on all three occasions tested.	Effects seen in C57BL/6J mice were similar to those observed previously in NMRI mice, implying that they are not strain specific[69].
Hexa-BDE	BDE-153 92.5% hexa-BDE; 7.5% hepta-BDE	NMRI mouse	0.45, 0.9, 9.0 mg/kg p.o. (single dose) on pnd10.	2,4 and 6 months after dosing	• No clinical signs of toxicity • Irreversible changes in spontaneous behaviour; both dose and time dependent. • Effects on learning and memory.	Decrease in nicotinic cholinergic receptors as measured by 3H-α-bungarotoxin binding. Cholinergic receptors have been implicated in mechanisms of learning and memory in other systems[57].
Octa-BDE	BDE-183 >98%	NMRI mouse	15.2 mg/kg p.o. (single dose) on pnd 3 or pnd10	2 months (spontaneous behavior) 3 months (swim maze)	• No clinical signs of toxicity. • No effects on spontaneous behavior or learning.	[70]
Octa-BDE	BDE-203 >98%	NMRI mouse	16.8 mg/kg p.o. (single dose) on pnd 3 or pnd10	24 hours (protein expression) 2 months (spontaneous behavior) 3 months (swim maze)	• No clinical signs of toxicity. • Disrupted habituation following dosing on pnd3 or pnd10. • Effects more marked if administered on pnd10 than pnd3. • Dosing on pnd10 adversely affected learning and memory. • Not seen if administered on pnd3.	Administration of BDE-203 or BDE-206 on pnd10 led to upregulation of the neuronal proteins CaMKII and synaptophysin, but only y ~1.5 fold. BDE-206 had no effect on the expression of GAP43 or tau proteins. Subtle effects such as these, seen 24 hours after dosing, cannot provide a mechanistic explanation for neurotoxic effects seen 2-3 months later[70,71].
Nona-BDE	BDE-206 >98%	NMRI mouse	18.5 mg/kg p.o. (single dose) on pnd 3 or pnd10		• No clinical signs of toxicity. • Disrupted habituation following dosing on pnd10 only.	

Deca-BDE	BDE-209 Purity not specified	NMRI mouse	1.34, 13.4, 20.1 mg/kg/day p.o. on pnd10 2.22, 20.1 mg/kg on pnd3 and pnd 19	2,4, and 6 months after dosing	• No clinical signs of toxicity. • Disturbances in spontaneous behavior • Only in mice dosed on pnd3, but observed at all testing times. • No evidence of neurotoxicity after dosing on pnd10 or pnd19.	Deca-BDE was absorbed and distributed to the brain (based on ^{14}C uptake following administration of radiolabelled deca-BDE. No accumulation in liver or heart. The authors conclude that the neurotoxic effect was due to a metabolite because it was observed only following dosing on pnd3, not pnd10, although the levels of parent compound were similar on the two days. However, no evidence is presented in support of this hypothesis[56].
Deca-BDE	BDE-209 >98%	NMRI mouse	20.1 mg/kg p.o. (single dose) on pnd3	Mice sacrificed 24h after dosing.	• No behavioural tests conducted	Subtle changes in expression of CaMKII, GAP-43 and BDNF, not sufficient to explain any biological effect which might occur several months later[72].
Deca-BDE	BDE-209 >98%	Sprague-Dawley rat	6.7, 20.1 mg/kg p.o. (single dose) on pnd3.	2 months after dosing	• No clinical sigs of toxicity. • Loss of habituation at 2 months of age. • Rats dosed at 20.1 mg/kg had the opposite response to nicotine compared with controls and those dosed at 6.7 mg/kg. At the high dose the rats had reduced activity immediately following a subcutaneous dose of nicotine, whereas the predicted effect (as seen in control sand low dose animals) was an increase in activity.	Effects in both rats and mice following administration on pnd3. No effect was observed in the mouse following dosing on pnd10 but this time of administration was not tested in rats. The effects observed following administration of nicotine implicate the cholinergic system in the effects of deca-BDE. The neurotoxic effects of deca-BDE appear to be restricted to the early postnatal period in the rat[73]
Deca-BDE	BDE-209 99.5% pure	C5BL/6J mouse	6, 20 mg/kg/day p.o. on pnd2-15	Functional test battery (pnd14, pnd16 and pnd20). Locomotor activity (pnd70, also at 1 yr of age).	• No effect on standard developmental endpoints. • In functional test battery, observed adverse effects on forelimb grip, and struggling behavior during handling on pnd 14/16. • Effects observed were inconsistent (often only seen at one time of testing) and tended to have disappeared by pnd20. • Locomotor activity reduced on pnd70. No effect observed when retested at 1 yr of age.	Dose-related reduction in serum T4, but only significant at the highest dose in males. The authors conclude that neonatal exposure to deca-BDE can affect some early neurobehavioural measures and locomotor activities in young adult male C57BL/6J mice, but do not draw any firm conclusions regarding the long-term consequences of these effects[74].
Deca-BDE	BDE-209 Purity not specified	BALB/c mouse (adults?)	300, 1000 mg/kg/ day for 4 weeks	Not specified	• Impairment of spatial learning and memory ability (Morris water maze and shuttle box).	Full paper not available online; limited detail available from abstract[75].

pnd: postnatal day

APPENDIX 6: SUMMARY OF "AN ORAL (GAVAGE) DEVELOPMENTAL NEUROTOXICITY STUDY OF DECABROMODIPHENYL OXIDE IN RATS"

1.1. OBJECTIVE

This oral gavage developmental neurotoxicity study in rats with decabromodiphenyl oxide was designed with the following objectives:

1) to determine the potential of the test substance to induce functional and/or morphological insult to the nervous system in the offspring of dams that were administered during pregnancy and lactation via oral gavage dosage levels of 1, 10, 100 or 1000 mg/kg/day;

2) To determine the concentration of the test substance in maternal and neonatal plasma and maternal milk samples on lactation/postnatal day 4 at dosage levels of 1 and 10 mg/kg/day. The concentration of the test substance in maternal and neonatal plasma and maternal milk resulting from oral (gavage) exposure of dams to the test substance in the same vehicle was already determined in a previous exposure assessment and dose range-finding developmental neurotoxicity study (Beck, 2009; WIL-635001) at dosage levels of 100, 300 and 1000 mg/kg/day.

1.2. STUDY DESIGN

Four groups of bred female Sprague Dawley Crl:CD (SD) rats (35/group; Groups 2-5) were administered daily by oral gavage the test substance (decabromodiphenyl oxide) in the vehicle corn oil from gestation day 6 through lactation day 21. Dosage levels were 1, 10, 100 and 1000 mg/kg/day. A concurrent control group of 35 rats (Group 1) received only the vehicle (corn oil) on a comparable regimen. The route of administration, the vehicle and the dosage levels selected for this study were based on the results of an exposure assessment and dose range-finding developmental neurotoxicity study in the same strain of rats (Beck, 2009; WIL-635001). F0 females were approximately 13 weeks of age at the beginning of test substance administration.

All animals were observed twice daily for appearance and behavior. Clinical observations were recorded daily (beginning on gestation day 0) and approximately 1 hour following dose administration during the treatment period. Body weights and food consumption were recorded at appropriate intervals during gestation and lactation. In addition, detailed clinical observations (DCO) were conducted out of the home cage for all dams in each group on gestation days 10 and 15 and on lactation days 10 and 21.

All F0 females were allowed to deliver and rear their offspring to lactation day 21, at which time the dams were euthanized and necropsied. F0 females that failed to deliver were necropsied on post-mating day 25. Clinical observations, body weights and gender identities were recorded for the F1 pups at appropriate intervals. On postnatal day (PND) 4, litters were culled (reduced) to 8 pups/litter (4 pups/sex, when possible). Following litter size reduction, 1 male and/or 1 female pup per litter were randomly assigned to one of the following evaluation subsets (A-D)

until the requisite sample size was met. Subset A of 30 pups/sex/group was assigned to DCO (PND 4, 11, 21, 35, 45 and 60), acoustic startle response (PND 20 and 60), locomotor activity (PND 13, 17, 21 and 61) and learning and memory (PND 62). A second subset (Subset B) of 20 pups/sex/group was selected for learning and memory (PND 22). A third subset (Subset C) of 15 pups/sex/group was selected for brain weight evaluations (PND 21), and from within this subset, 10 pups/sex from the control and 1000 mg/kg/day groups were designated for neuropathology and morphometry evaluations (PND 21). Furthermore, 10 pups/sex/group assigned to Subset A and 5 pups/sex/group assigned to Subset B (representing as many litters as possible) were further assigned to Subset D for brain weight evaluations (PND 72). Within Subset D, 10 pups/sex from the control and 1000 mg/kg/day groups were designated for neuropathology and morphometry evaluations (PND 72). In addition, 10 males each from the 1, 10 and 100 mg/kg/day groups that were assigned to Subset D were later selected for PND 72 morphometry evaluation. The remaining 20 pups/sex/group in Subset A that were not selected for PND 72 evaluation were assigned to PND 120 and 180 locomotor activity. A nicotine challenge was conducted during the PND 61, 120 and 180 locomotor activity assessments. Animals were administered nicotine (20 [PND 61] or 10-11 [PND 120 and 180] pups/sex/group) or saline (9-10 pups/sex/group [PND 61, 120 and 180]) following the initial 60-minute test session, and then subjected to a second 60-minute test session.

Indicators of physical development (balanopreputial separation and vaginal patency) were evaluated for F1 animals assigned to Subsets A and B. All F1 animals not selected for behavioral evaluations were euthanized and necropsied on PND 21. F1 animals selected for learning and memory assessment on PND 22, and not selected for PND 72 evaluation, and F1 animals selected for locomotor activity assessment on PND 120 and 180 were necropsied following completion of these respective assessments.

An additional bioanalytical phase, composed of 2 groups of bred female rats (8 rats/group), was conducted to determine the concentration of the test substance in maternal and neonatal plasma and maternal milk. These females were administered the test substance daily from gestation day 6 through lactation day 4 at the 2 lowest dosage levels (1 and 10 mg/kg/day). Clinical observations and body weights for the F0 females were recorded at appropriate intervals. Clinical observations, body weights and gender identities were recorded for the F1 pups at appropriate intervals. Blood samples were collected from 4 F0 females/group and their F1 litters at 8 hours after dose administration on lactation day/PND 4. The remaining 4 F0 females/group had milk collected 8 hours following dose administration on lactation day 4. All F0 dams and F1 pups were euthanized following sample collection and discarded. One F0 female in the bioanalytical phase that failed to deliver was euthanized on post-mating day 25 and discarded without examination.

1.3. RESULTS

Mean concentrations of the test substance in maternal and pup plasma on lactation day/PND 4 at 10 mg/kg/day were 1700 and 2140 ng/mL, respectively. These concentrations were similar to those noted in a previous preliminary range-finding study (Beck, 2009; WIL-635001), in which maternal and pup plasma concentrations were 1334 to 1812 ng/mL and 1998 to 2535 ng/mL, respectively, at the 3 dosage levels used (100,300 and 1000 mg/kg/day). Based on these results, no differences were apparent when comparing plasma concentrations (internal

exposures) between the external dosage levels of 10 to 1000 mg/kg/day. Mean concentrations of the test substance in maternal and pup plasma at 1 mg/kg/day (510 and 929 ng/mL, respectively) were at least 3- and 2-fold lower, respectively, than those noted at 10 mg/kg/day. Mean concentrations of the test substance in maternal milk samples at 1 and 10 mg/kg/day on lactation day 4 were 509 and 1250 ng/mL, respectively. These values were similar to those noted in the previous preliminary range-finding study (Beck, 2009; WIL-635001) at dosage levels of 100 to 1000 mg/kg/day (574 to 628 ng/mL).

There were no test substance-related mortalities in the F0 maternal animals during the study. There were no test substance-related clinical findings noted during the daily examinations or at approximately 1 hour following dose administration. Maternal detailed clinical observation parameters, as well as maternal body weights and food consumption during gestation and lactation, were unaffected by test substance administration.

There were no maternal tests substance-related differences noted between groups when comparing the mean length of gestation, the process of parturition and the internal macroscopic findings. The mean numbers of former implantation sites and unaccounted-for sites, as well as the mean number of pups born, live litter size and the percentage of males at birth were similar across all groups.

There were no test substance-related effects on F1 postnatal survival, clinical observations, body weights, body weight gains or necropsy findings at any dosage level. In addition, startle responsiveness, swimming ability, learning and memory and attainment of sexual developmental landmarks (balanopreputial separation and vaginal patency) were unaffected by maternal test substance administration. There were no test substance-related effects on locomotor activity, which was evaluated on PND 13, 17, 21, 61, 120 and 180.

There were no test substance-related effects on brain weights, lengths or widths on PND 21 and 72. There were no histopathological alterations related to maternal test substance administration in PND 21 and PND 72 brains or PND 72 central or peripheral nervous system tissues. There were no differences in morphometric measurements that were considered related to test substance administration.

1.4. CONCLUSIONS

There was no evidence of maternal toxicity at any dosage level of decabromodiphenyl oxide evaluated in this study. Additionally, there were no effects on offspring survival and growth, or on any of the neurobehavioral parameters evaluated in this study. Normal patterns of habituation were observed at all relevant ages tested for both locomotor activity and auditory startle response. Therefore, under the conditions of this study, no evidence of developmental neurotoxicity was observed at any dosage level evaluated. Thus, the no-observed-adverse-effect level (NOAEL) for both F0 systemic toxicity and F1 neonatal and developmental neurotoxicity of decabromodiphenyl oxide was considered to be 1000 mg/kg/day, the highest dosage level administered in this study. Based on the mean plasma concentration of the test substance in F1 pups at 8 hours following maternal dose administration on lactation day 4, the internal exposure at dosage levels between 10 and 1000 mg/kg/day was determined in this study and a previous study (Beck, 2009; WIL-635001) to be 1998 ng/mL to 2535 ng/mL.

REFERENCES

1. Tokarz, J.A., 3rd, et al. Reductive debromination of polybrominated diphenyl ethers in anaerobic sediment and a biomimetic system. *Environ Sci Technol*, 2008. 42(4): p. 1157-64.
2. Klosterhaus, S.L. and J.E. Baker. Bioavailability of decabromodiphenyl ether to the marine polychaete *Nereis virens Environ Toxicol Chem*, 2010. 29: p. 860-868.
3. Kajiwara, N., Y. Noma, and H. Takigami. Photolysis studies of technical decabromodiphenyl ether (DecaBDE) and ethane (DeBDethane) in plastics under natural sunlight. *Environ Sci Technol*, 2008. 42(12): p. 4404-9.
4. Stapleton, H.M. and N.G. Dodder. Photodegradation of decabromodiphenyl ether in house dust by natural sunlight. *Environ Toxicol Chem*, 2008. 27(2): p. 306-12.
5. Christiansson, A., et al. Identification and quantification of products formed via photolysis of decabromodiphenyl ether. *Environ Sci Pollut Res Int*, 2009. 16(3): p. 312-21.
6. Suh, Y.W., et al. UVA/B-induced formation of free radicals from decabromodiphenyl ether. *Environ Sci Technol*, 2009. 43(7): p. 2581-8.
7. Mas, S., et al. Photodegradation study of decabromodiphenyl ether by UV spectrophotometry and a hybrid hard- and soft-modelling approach. *Anal Chim Acta*, 2008. 618(1): p. 18-28.
8. Shih, Y.H. and C.K. Wang. Photolytic degradation of polybromodiphenyl ethers under UV-lamp and solar irradiations. *J Hazard Mater*, 2009. 165(1-3): p. 34-8.
9. Eriksson, J., et al. Photochemical decomposition of 15 polybrominated diphenyl ether congeners in methanol/water. *Environ Sci Technol*, 2004. 38(11): p. 3119-25.
10. Xie, Q., et al. Important role of reaction field in photodegradation of deca-bromodiphenyl ether: theoretical and experimental investigations of solvent effects. *Chemosphere*, 2009. 76(11): p. 1486-90.
11. Davis, E.F. and H.M. Stapleton. Photodegradation pathways of nonabrominated diphenyl ethers, 2-ethylhexyltetrabromobenzoate and di(2-ethylhexyl)tetrabromophthalate: identifying potential markers of photodegradation. *Environ Sci Technol*, 2009. 43(15): p. 5739-46.
12. Soderstrom, G., et al. Photolytic debromination of decabromodiphenyl ether (BDE 209). *Environ Sci Technol*, 2004. 38(1): p. 127-32.
13. Keum, Y.S. and Q.X. Li. Reductive debromination of polybrominated diphenyl ethers by zerovalent iron. *Environ Sci Technol*, 2005. 39(7): p. 2280-6.
14. Shih, Y.H. and Y.T. Tai. Reaction of decabrominated diphenyl ether by zerovalent iron nanoparticles. *Chemosphere*. 78(10): p. 1200-6.
15. Ahn, M.Y., et al. Birnessite mediated debromination of decabromodiphenyl ether. *Chemosphere*, 2006. 64(11): p. 1801-7.
16. Gerecke, A.C., et al. Anaerobic degradation of decabromodiphenyl ether. *Environ Sci Technol*, 2005. 39(4): p. 1078-83.
17. Gerecke, A.C., et al. Anaerobic degradation of brominated flame retardants in sewage sludge. *Chemosphere*, 2006. 64(2): p. 311-7.
18. He, J., K.R. Robrock, and L. Alvarez-Cohen. Microbial reductive debromination of polybrominated diphenyl ethers (PBDEs). *Environ Sci Technol*, 2006. 40(14): p. 4429-34.
19. Rayne, S., M.G. Ikonomou, and M.D. Whale. Anaerobic microbial and photochemical degradation of 4,4'-dibromodiphenyl ether. *Water Res*, 2003. 37(3): p. 551-60.
20. Welsh, G., *Polybrominated diphenyl ethers: soil sorption and microbial degradation*, in *Department of Biolgocial Sciences*. 2008, University of Cincinnati: Cincinnati.
21. Vonderheide, A.P., et al. Rapid breakdown of brominated flame retardants by soil microorganisms. *Journal of Analytical Spectrometry*, 2006. 21: p. 1232-1239.
22. Rodbrock, K.R., P. Korytar, and L. Alvarez-Cohen. Pathways for the anaerobic microbial debromination of polybrominated diphenyl ethers. *Env. Sci. Technol.* , 2008. 42: p. 2845-2852.
23. Zhou, J., et al. Effect of Tween 80 and beta-cyclodextrin on degradation of decabromodiphenyl ether (BDE-209) by White rot fungi. *Chemosphere*, 2007. 70(2): p. 172-7.
24. Joner, E.J., et al. Rhizosphere effects on microbial community structure and dissipation and toxicity of polycyclic aromatic hydrocarbons (PAHs) in spiked soil. *Environ Sci Technol*, 2001. 35(13): p. 2773-7.
25. Nyholm, J.R., C. Lundberg, and P.L. Andersson. Biodegradation kinetics of selected brominated flame retardants in aerobic and anaerobic soil. *Environ Pollut*, 2010. 158(6): p. 2235-40.
26. Huang, H., et al. Behavior of decabromodiphenyl ether (BDE-209) in the soil-plant system: uptake, translocation, and metabolism in plants and dissipation in soil. *Environ Sci Technol*, 2010. 44(2): p. 663-7.
27. Wilford, B.H., et al. Decabromodiphenyl ether (deca-BDE) commercial mixture components, and other PBDEs, in airborne particles at a UK site. *Environ Int*, 2008. 34(3): p. 412-9.
28. Leslie, H.A., et al., *DECAMONITOR: Monitoring of decabromodiphenyl ether in the environment: Birds eggs, sewage sludge and sediments. Final report for the third sampling year (2007).* , in *Confidential progress report produced for Bromine Science and Environmental Forum (BSEF), 31st July*. 2008.
29. Leslie, H.A., et al., *DECAMONITOR: Monitoring of decabromodiphenyl ether in the environment: Final report for the fourth sampling year (2008)* in *Confidential progress report produced for Bromine Science and Environmental Forum (BSEF), 27th April*. 2009.

30. Chen, D., et al. Polybrominated diphenyl ethers in peregrine falcon (Falco peregrinus) eggs from the northeastern U.S. *Environ Sci Technol*, 2008. 42(20): p. 7594-600.
31. Cheung, K.C., et al. Exposure to polybrominated diphenyl ethers associated with consumption of marine and freshwater fish in Hong Kong. *Chemosphere*, 2008. 70(9): p. 1707-20.
32. Christiansson, A., et al. Polybrominated diphenyl ethers in aircraft cabins--a source of human exposure? *Chemosphere*, 2008. 73(10): p. 1654-60.
33. Stapleton, H.M., et al. Determination of polybrominated diphenyl ethers in indoor dust standard reference materials. *Anal Bioanal Chem*, 2006. 384(3): p. 791-800.
34. Kohler, M., et al. Temporal trends, congener patterns, and sources of octa-, nona-, and decabromodiphenyl ethers (PBDE) and hexabromocyclododecanes (HBCD) in Swiss lake sediments. *Environ Sci Technol*, 2008. 42(17): p. 6378-84.
35. Holden, A., et al. Unusual hepta- and octabrominated diphenyl ethers and nonabrominated diphenyl ether profile in California, USA, peregrine falcons (Falco peregrinus): more evidence for brominated diphenyl ether-209 debromination. *Environ Toxicol Chem*, 2009. 28(9): p. 1906-11.
36. La Guardia, M.J., R.C. Hale, and E. Harvey. Evidence of debromination of decabromodiphenyl ether (BDE-209) in biota from a wastewater receiving stream. *Environ Sci Technol*, 2007. 41(19): p. 6663-70.
37. el Dareer, S.M., et al. Disposition of decabromobiphenyl ether in rats dosed intravenously or by feeding. *J Toxicol Environ Health*, 1987. 22(4): p. 405-15.
38. Morck, A., et al. Decabromodiphenyl ether in the rat: absorption, distribution, metabolism, and excretion. *Drug Metab Dispos*, 2003. 31(7): p. 900-7.
39. Sandholm, A., B.M. Emanuelsson, and E.K. Wehler. Bioavailability and half-life of decabromodiphenyl ether (BDE-209) in rat. *Xenobiotica*, 2003. 33(11): p. 1149-58.
40. Riu, A., et al. Disposition and metabolic profiling of [14C]-decabromodiphenyl ether in pregnant Wistar rats. *Environ Int*, 2008. 34(3): p. 318-29.
41. Van der Ven, L.T., et al. A 28-day oral dose toxicity study in Wistar rats enhanced to detect endocrine effects of decabromodiphenyl ether (decaBDE). *Toxicol Lett*, 2008. 179(1): p. 6-14.
42. Huwe, J.K. and D.J. Smith. Accumulation, whole-body depletion, and debromination of decabromodiphenyl ether in male sprague-dawley rats following dietary exposure. *Environ Sci Technol*, 2007. 41(7): p. 2371-7.
43. Carlson, G.P. Induction of xenobiotic metabolism in rats by brominated diphenyl ethers administered for 90 days. *Toxicol Lett*, 1980. 6(3): p. 207-12.
44. Carlson, G.P. Induction of xenobiotic metabolism in rats by short-term administration of brominated diphenyl ethers. *Toxicol Lett*, 1980. 5(1): p. 19-25.
45. Pacyniak, E.K., et al. The flame retardants, polybrominated diphenyl ethers, are pregnane X receptor activators. *Toxicol Sci*, 2007. 97(1): p. 94-102.
46. Bruchajzer, E., et al. Toxicity of penta- and decabromodiphenyl ethers after repeated administration to rats: a comparative study. *Arch Toxicol*. 84(4): p. 287-99.
47. Van den Steen, E., et al. Accumulation, tissue-specific distribution and debromination of decabromodiphenyl ether (BDE 209) in European starlings (Sturnus vulgaris). *Environ Pollut*, 2007. 148(2): p. 648-53.
48. Stapleton, H.M., et al. Debromination of the flame retardant decabromodiphenyl ether by juvenile carp (Cyprinus carpio) following dietary exposure. *Environ Sci Technol*, 2004. 38(1): p. 112-9.
49. Tomy, G.T., et al. Bioaccumulation, biotransformation, and biochemical effects of brominated diphenyl ethers in juvenile lake trout (Salvelinus namaycush). *Environ Sci Technol*, 2004. 38(5): p. 1496-504.
50. Stapleton, H.M., et al. In vivo and in vitro debromination of decabromodiphenyl ether (BDE 209) by juvenile rainbow trout and common carp. *Environ Sci Technol*, 2006. 40(15): p. 4653-8.
51. Kierkgaard, A., et al. Dietary Uptake and Biological Effects of Decabromodiphenyl Ether in Rainbow Trout (Oncorhynchus mykiss). . *Environ. Sci. Technol.*, 1999. 33: p. 1612-1617.
52. Nyholm, J.R., et al. Uptake and biotransformation of structurally diverse brominated flame retardants in zebrafish (Danio rerio) after dietary exposure. *Environ Toxicol Chem*, 2009. 28(5): p. 1035-42.
53. Kuo, Y.M., et al. Bioaccumulation and biotransformation of decabromodiphenyl ether and effects on daily growth in juvenile lake whitefish (Coregonus clupeaformis). *Ecotoxicology*. 19(4): p. 751-60.
54. Ciparis, S. and R.C. Hale. Bioavailability of polybrominated diphenyl ether flame retardants in biosolids and spiked sediment to the aquatic oligochaete, Lumbriculus variegatus. *Environ Toxicol Chem*, 2005. 24(4): p. 916-25.
55. Sellstrom, U., et al. Effect of sewage-sludge application on concentrations of higher-brominated diphenyl ethers in soils and earthworms. *Environ Sci Technol*, 2005. 39(23): p. 9064-70.
56. Viberg, H., et al. Neurobehavioral derangements in adult mice receiving decabrominated diphenyl ether (PBDE 209) during a defined period of neonatal brain development. *Toxicol Sci*, 2003. 76(1): p. 112-20.
57. Viberg, H., A. Fredriksson, and P. Eriksson. Neonatal exposure to polybrominated diphenyl ether (PBDE 153) disrupts spontaneous behaviour, impairs learning and memory, and decreases hippocampal cholinergic receptors in adult mice. *Toxicol Appl Pharmacol*, 2003. 192(2): p. 95-106.
58. Kuo, Y.M., et al. Bioaccumulation and biomagnification of polybrominated diphenyl ethers in a food web of Lake Michigan. *Ecotoxicology*. 19(4): p. 623-34.
59. Akutsu, K., et al. GC/MS analysis of polybrominated diphenyl ethers in fish collected from the Inland Sea of Seto, Japan. *Chemosphere*, 2001. 44(6): p. 1325-33.

60. Sormo, E.G., et al. Biomagnification of polybrominated diphenyl ether and hexabromocyclododecane flame retardants in the polar bear food chain in Svalbard, Norway. *Environ Toxicol Chem*, 2006. 25(9): p. 2502-11.
61. Burreau, S., et al. Biomagnification of PBDEs and PCBs in food webs from the Baltic Sea and the northern Atlantic Ocean. *Sci Total Environ*, 2006. 366(2-3): p. 659-72.
62. Law, R.J., et al. Levels and trends of brominated flame retardants in the European environment. *Chemosphere*, 2006. 64(2): p. 187-208.
63. Law, K., et al. Bioaccumulation and trophic transfer of some brominated flame retardants in a Lake Winnipeg (Canada) food web. *Environ Toxicol Chem*, 2006. 25(8): p. 2177-86.
64. Eljarrat, E., et al. Decabrominated diphenyl ether in river fish and sediment samples collected downstream an industrial park. *Chemosphere*, 2007. 69(8): p. 1278-86.
65. Vives, I., et al. Polybromodiphenyl ether flame retardants in fish from lakes in European high mountains and Greenland. *Environ Sci Technol*, 2004. 38(8): p. 2338-44.
66. Song, W., et al. Polybrominated diphenyl ethers in the sediments of the Great Lakes. 2. Lakes Michigan and Huron. *Environ Sci Technol*, 2005. 39(10): p. 3474-9.
67. Burreau, S., et al. Biomagnification of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) studied in pike (Esox lucius), perch (Perca fluviatilis) and roach (Rutilus rutilus) from the Baltic Sea. *Chemosphere*, 2004. 55(7): p. 1043-52.
68. Wang, Y., et al. Effect of municipal sewage treatment plant effluent on bioaccumulation of polychlorinated biphenyls and polybrominated diphenyl ethers in the recipient water. *Environ Sci Technol*, 2007. 41(17): p. 6026-32.
69. Viberg, H., A. Fredriksson, and P. Eriksson. Investigations of strain and/or gender differences in developmental neurotoxic effects of polybrominated diphenyl ethers in mice. *Toxicol Sci*, 2004. 81(2): p. 344-53.
70. Viberg, H., et al. Neonatal exposure to higher brominated diphenyl ethers, hepta-, octa-, or nonabromodiphenyl ether, impairs spontaneous behavior and learning and memory functions of adult mice. *Toxicol Sci*, 2006. 92(1): p. 211-8.
71. Viberg, H. Exposure to polybrominated diphenyl ethers 203 and 206 during the neonatal brain growth spurt affects proteins important for normal neurodevelopment in mice. *Toxicol Sci*, 2009. 109(2): p. 306-11.
72. Viberg, H., W. Mundy, and P. Eriksson. Neonatal exposure to decabrominated diphenyl ether (PBDE 209) results in changes in BDNF, CaMKII and GAP-43, biochemical substrates of neuronal survival, growth, and synaptogenesis. *Neurotoxicology*, 2008. 29(1): p. 152-9.
73. Viberg, H., A. Fredriksson, and P. Eriksson. Changes in spontaneous behaviour and altered response to nicotine in the adult rat, after neonatal exposure to the brominated flame retardant, decabrominated diphenyl ether (PBDE 209). *Neurotoxicology*, 2007. 28(1): p. 136-42.
74. Rice, D.C., et al. Developmental delays and locomotor activity in the C57BL6/J mouse following neonatal exposure to the fully-brominated PBDE, decabromodiphenyl ether. *Neurotoxicol Teratol*, 2007. 29(4): p. 511-20.
75. Zhai, J.X., et al. [Effect of exposure to higher decabrominated diphenyl ether (PBDE-209) on learning and memory functions of BALB/c mice]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi*. 28(1): p. 25-9.