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Risk of Combination Effects Between Decabromodiphenyl Ether and Other Polybrominated Diphenyl Ethers

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Risk of Combination Effects Between Decabromodiphenyl Ether and Other Polybrominated Diphenyl Ethers.
Risiko for toksikologiske interaksjoner mellom dekabromdifenyleter og andre polybromertedifenyletere

Summary - sammendrag

Decabromodiphenyl ether BDE-209 acts as a slow-relase reeservoir for lower brominated, more toxic PBDEs, via abiotic and biotic transformation processes. As a result, human populations and biota worldwide are exposed to BDE-209 and other PBDEs in combination. Young children experience the highest exposures of all age groups.
While the critical toxicity of BDE-209 to humans is judged to be developmental neurotoxicity, the effects in biota are more varied. Plants and algae do not appear to be sensitive, but molluscs react with DNA damage. In fish and amphibians, effects attributable to thyroid disruption occur.
It is plausibel to expect combination effects of BDE-209 and other congeners in terms of developmental neurotoxicity. An assessment of combined human exposures revealed that tolerable exposures are exceeded for all age groups, but particularly for small children, which warrants health concerns. A scoping mixture risk assessment for environmental scenarios revealed concerns for top predators, especially polar bears.

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4 subject words

Mixture risk assessment; decabromodiphenyl ether; biotransformation, combined exposures

Foreword

This report on the risk of combination effects between decabromodiphenyl ether and other polybrominated diphenyl ethers was commissioned by the Norwegian Environmental Protection Agency. Brunel University, Institute for the Environment was granted the contract through competitive tendering.

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Front page image: Wordle graphic showing the frequency with which each PBDE congener was examined in scientific studies, word size is proportional to the number of studies that examined each congener using a Log scale from 1 study (e.g. BDE-18) to 486 studies (BDE-209).

London, December 2013

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Contents

1. Summary

The purpose of this report was to compile and review scientific literature and government reports relevant to the risk, likelihood and type of combination effects between decabromodiphenyl ether (BDE-209) and other polybrominated diphenyl ethers (PBDEs) in biota. The report may support Norway's nomination of the commercial BDE-209 mixture (c-decaBDE) to the Stockholm Convention on Persistent Organic Pollutants (POPs) and the review of BDE-209 by the Persistent Organic Pollutant Review Committee (POPRC) in particular, for the purposes of considering toxicological interactions as part of a hazard assessment under the Stockholm Convention (Annex E, paragraph b).

A literature database with 995 records dating from 1987 to 2013 was assembled. A search for reports by various regulatory authorities was also carried out and 59 reports were identified. The database was evaluated for a narrative review of the literature with a focus on four relevant topics: Biotransformation of BDE-209, mixtures of BDE-209 with other PBDEs to which biota are exposed, including as a result of biotransformation, toxic effects for mixtures of BDE-209 and other PBDEs, and a description of mixture risk and factors influencing risk, with an assessment of the impact of data gaps and the use of bridging concepts. The narrative review is organised into separate strands for human toxicology and the environment, and also contains the information used to support case studies of PBDE mixture risk assessment.

Human biomonitoring studies provide convincing evidence that BDE-209 debromination and oxidation reactions first identified in experimental studies with rodents also occur in humans. In addition, humans are exposed to congeners with even fewer bromine atoms that originate from abiotic and biotic BDE-209 transformations. Through abiotic and biotic processes, BDE-209 is transformed to PBDEs with as few as three bromine atoms. These lower brominated congeners are usually more toxic than BDE-209.

Human populations worldwide are exposed to combinations of PBDEs. Exposure in foetal life occurs through trans-placental transfer from the mother, in neonatal life through breast feeding, and during later life stages via food, especially fish, and ingestion of dust. BDE-209 alone contributes approximately half of the total PBDE exposure of young children and adults. Other dominant BDE congeners include BDE-47, -99, -100 and -153. On a body weight basis, young children of age 6 month to 3 years experience the highest PBDE exposures of all age groups.

The occurrence of PBDEs in environmental media and biota was investigated for 76 scenarios, grouped into three major classes, pelagic, benthic and predators. A wide variety of PBDEs occurs in all media and biota. In water and sediments (freshwater and marine), soil, microorganisms, plants and invertebrates, BDE-209 is the dominant of all PBDE congeners. In fish and mammals, BDE-47 is more prevalent, while amphibians show high levels of BDE-99. In birds, BDE-47, -99 and -209 are the predominant congeners.

The critical toxicity of BDE-209 and other PBDEs to mammals and humans is judged to be developmental neurotoxicity and is thought to arise through disruption of thyroid hormones and / or direct toxicity to neuronal cells. The toxicity of BDE-209 and other PBDEs to biota is more varied. While plants and algae do not appear to be sensitive, molluscs can react with DNA damage. In fish and amphibians, effects attributable to thyroid disruption occur.

While the combined effects of BDE-209 with other PBDEs have not been studied experimentally, a combination of BDE-47 and -99 showed synergistic cytotoxic effects to neuronal cells. On the basis of common modes of action and common adverse outcomes, it can be expected that BDE-209 and other PBDEs produce combined developmental neurotoxicity. The joint effects of PBDEs for exposure scenarios relevant to humans were evaluated in a case study by using the Hazard Index approach within the tiered WHO / IPCS assessment framework. Tolerable combined exposures are exceeded for all age groups, particularly for small children which warrants health concerns. A scoping mixture risk assessment for environmental scenarios revealed concerns for top predators, especially polar bears.

In conclusion, BDE-209 is a source of more toxic, lower brominated PBDEs which have the capacity to work together with BDE-209 to produce combined toxicity. An evaluation of BDE-209 in isolation, without taking account of such combination effects, would significantly underestimate the toxicity of BDE-209. Combined

exposures to BDE-209 and other PBDE pose significant health concerns, especially for young children of age 6 month to 3 years which bear the highest PBDE exposures of all age groups.

2. Introduction & background

2.1 Introduction

The purpose of this report was to compile and review scientific literature and government reports relevant to the risk, likelihood and type of toxicological interactions between decabromodiphenyl ether and other polybrominated diphenyl ethers (PBDEs) in biota. Throughout this report the abbreviation c-decaBDE is used to refer to commercial decabromodiphenyl ether and BDE-209 is used to refer to the single, fully brominated decabromodiphenyl ether congener.

The report may support Norway's nomination of c-decaBDE to the Stockholm Convention on Persistent Organic Pollutants (POPs) and the review of c-decaBDE by the Persistent Organic Pollutant Review Committee (POPRC); in particular, for the purposes of considering toxicological interactions as part of a hazard assessment under the Stockholm Convention (Annex E, paragraph b). In order to suit the purposes of the Stockholm Convention which requires assessment of human toxicological as well as ecotoxicological aspects, the report encompasses both human/mammalian and ecotoxicology,

This report is the final report for a project financed by the Norwegian Environment Agency; procurement no. 3013043; project term: October-December 2013.

2.2 Background

Persistent organic pollutants (POPs) are a group of chemicals that can spread over wide geographical distances, have long environmental half-lives, accumulate in food chains and are toxic to living organisms. These properties mean that POPs pose a threat to human health and the environment. The Stockholm convention is a global treaty that bans or strictly controls the production, trade and use of POPs in order to protect human health and the environment. In May 2013, Norway proposed the inclusion of the commercial BDE-209 mixture (c-decaBDE) in the convention. In October 2013 the Ninth meeting of the expert committee of the Stockholm Convention reviewed the proposal and decided, in accordance with paragraph 4(a) of Article of the Convention, that it was satisfied that the screening criteria set out in Annex D to the Convention were fulfilled. An ad hoc working group was established to review the proposal further and to prepare a draft risk profile in accordance with Annex E to the Convention intersessionally. The purpose of the risk profile is to evaluate whether c-decaBDE is likely, as a result of its long-range environmental transport, to lead to significant adverse human health and/ or environmental effects, such that global action is warranted. For this purpose the risk profile should include, but is not limited to, consideration of 1) evidence of adverse effects to human health or to the environment that justifies consideration of the chemical within the scope of the Convention; 2) toxicity or ecotoxicity data that indicate the potential for damage to human health or the environment and 3) information on hazard for endpoints or endpoints of concern, including a consideration of toxicological integrations involving multiple chemicals.

Polybrominated diphenyl ethers (PBDEs) are a class of brominated aromatic compounds with a basic structure consisting of two phenyl rings linked by an ether bond (EFSA, 2011). There are 209 possible PBDE congeners, which differ in the number and position of the bromine atoms in the two phenyl rings. PBDEs form the same number of congeners and the substitution patterns are identical to the congeners of polychlorinated biphenyls (PCBs). Hence, the PBDEs share the same congener numbering system as proposed for PCBs (Ballschmiter and Zell, 1980). The focus of this report is the fully brominated decabrominated diphenyl ether, BDE-209, which is the main component of c-decaBDE. Two other commercial PBDE products have already been listed as POPs in the Stockholm Convention: commercial pentabromodiphenyl ether (which contains mainly tetra and penta brominated congeners) and commercial octabromodiphenyl ether (which contains hexa and hepta brominated congeners). The main congeners in these products include BDE-47 (tetra), BDE-99 (penta), BDE-153 (hexa), BDE-154 (hexa), BDE-175 (hepta), BDE-183 (hepta).

C-decaBDE is a synthetic mixture of PBDE congeners consisting of mainly decabromodiphenyl ether (BDE-209) (≥97%), with small amounts of other brominated diphenyl ethers such as nonabromodiphenyl ether and octabromodiphenyl ether. Historically a range of 77.4-98 % decaBDE, and smaller amounts of the congeners of nonaBDE (0.3-21.8 %) and octaBDE (0-0.04 %) has been reported (ECHA 2012). Total tri-, tetra-, penta-, hexa- and heptaBDEs are typically present at concentrations below 0.0039 % w/w (ECHA 2012). C-decaBDE is widely used as an additive flame retardant in plastics and textiles (BSEF, 2013). In plastics, c-decaBDE is used for electrical and electronic equipment, including the housings of computers and TV sets; in vehicles, for example planes, cars and trucks; and in construction and building (i.e. wires and cables, pipes and carpets). In textile applications, Deca-BDE is used in contract textiles (curtain, blinds, textile wall coverings and seating fabrics), mainly for public buildings and transport; and in domestic furniture textiles (BSEF, 2013).

C-decaBDE can act on the same biological endpoints and induce the same/similar adverse effects as other PBDEs (UNEP, 2013). BDE-209, the main component of c-decaBDE, may produce toxic effects either alone or in concert with metabolic degradation products. Humans and wildlife are exposed to PBDE mixtures as a result of 1) the presence of multiple PBDE congeners in the environment and 2) biotransformation within an organism which may convert BDE-209 to lower brominated PDBE congeners, including some that are already listed POPs (tetra, penta, hexa and hepta BDEs in commercial penta and octa BDE products.

2.3 Report structure

This report begins with a description of the detailed search strategy used to identify relevant literature and reports (section 3) together with a brief summary of the literature that was identified (section 4). The main body of the report is a narrative review (section 5) of the identified literature relevant to four topics:

1. Biotransformation of BDE-209
2. Likely mixtures of BDE-209 with other PBDEs to which biota are exposed, including as a result of biotransformation
3. Toxic effects possible for mixtures of BDE-209 and other PBDEs
4. Description of mixture risk and factors influencing risk, including assessment of the impact of data gaps and the use of bridging concepts

The narrative review is organised into separate strands for human toxicology and the environment, and also contains the information used to support a mixture risk assessment (Section 6). The report finishes with a discussion that unifies the human and environmental strands of the report.

2.4 References

Ballschmiter, K. & Zell, M. 1980, "Baseline Studies of the Global Pollution .1. Occurrence of Organohalogens in Pristine European and Antarctic Aquatic Environments", *International Journal of Environmental Analytical Chemistry*, vol. 8, no. 1, pp. 15-35.

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EFSA, European Food Standards Authority (2011). EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on Polybrominated Diphenyl Ethers (PBDEs) in Food. EFSA Journal 2011;9(5):2156. [274 pp.] doi:10.2903/j.efsa.2011.2156. Available online: www.efsa.europa.eu/efsajournal

UNEP (2013). Proposal to list decabromodiphenyl ether (commercial mixture, c-BDE-209) in Annexes A, B and/or C to the Stockholm Convention on Persistent Organic Pollutants. Persistent Organic Pollutants Review Committee. Ninth meeting. Rome, 14–18 October 2013. UNEP/POPS/POPRC.9/2

3. Methods & procedures

3.1 Search strategy for scientific literature

The systematic literature search strategy is detailed in Box 1, in addition to the terms proposed in the project tender, four terms were added (indicated with an asterisk in Box 1) as a result of scoping searches that identified their usefulness. The search was performed on 14th October 2013 and the results were imported into a Microsoft Access database for the purposes of the project team. An additional synonym ("decabromodiphenyl oxide") was searched on 13th November 2013, and 12 results added to the database.

Box 1: systematic literature search strategy

Search terms (entered in Web of Knowledge field 'topic'):
"2,3,4,5,6-Pentabromo-1-(2,3,4,5,6-pentabromophenoxy)benzene"
decabromodiphenylether
decabrominated diphenyl ether
decabrominated diphenyl ethers
decabromodiphenyl oxide*
decaBDE
deca-BDE
BDE-209
BDE209
"BDE 209"**
PBDE-209
PBDE209
1163-19-5
deca-BDEs**
decaBDEs*
"deca BDEs"**

Timespan:	All years
Search language:	English
Database:	Web of Knowledge (including Web of Science and MEDLINE/PubMed)
Note(s):	All terms were joined with "OR"
Results:	983 results, 14th October 2013 12 results added, 13th November 2013

* additional synonym added 13.11.2013 after the main search was completed
** terms added as a result of scoping searches

3.2 Search strategy for grey literature

The search for government reports and other so-called grey literature followed several strands. The first step was to collect all such literature cited in the Proposal to list decabromodiphenyl ether in Annexes A, B and/or C to the Stockholm Convention on Persistent Organic Pollutants (UNEP, 2013).

A second exploratory strand was also followed by running a Google search with 'PBDE' as the search term. The first ten pages of results of this Google search were scanned for relevant pages or documents. Relevant documents were collated and then scanned for the term 'BDE' to check whether the publication contained any information of relevance to this project.

Finally, this was completed by searching the European Commission's Joint Research Centre repository for any document containing the term 'PBDE'. This yielded 69 hits. The word 'BDE' was then looked up in these 69 documents to check whether they contained any information of relevance to the current project.

3.3 Database structure

A database was built to contain all the unique results from the defined search, and was organised to reflect the major project interests.

Each record was classified as to whether it had minor relevance to the project aims (using a defined list). Relevant records were then classified as their relevance to either human or eco-toxicology, their bibliographic details (journal article, review, other publication type etc) and key experimental parameters (study type, species, geographic location etc). Finally each record was classified by its relevance to three specified project interests:

- biotransformation of BDE-209, with biomonitoring or scientific data
- the likely composition of PBDE mixtures including BDE-209, that can be expected
- hazard or effect data for BDE-209

Classification results for each article are provided in an extensive database report (Appendix A).

3.3.1 Congener indexing

All studies identified as having exposure data for humans, other organisms or environmental compartments were indexed by each congener studied. This identified all the studies that have examined a given congener, in preparation for data collection and synthesis of the reported PBDE levels. Non-PBDE chemicals were not indexed.

4. Results of systematic literature search

4.1 Literature database content report

The search produced 995 records, of which 12 were identified as duplicates and removed from the database. The remaining 983 unique records were then classified further in a Microsoft Access database, referred to as DECA DB. One additional scientific publication was identified by searching the citation lists of major reports for publications not identified by the systematic search, resulting in a final database of 984 unique records. The results of classification of this literature are now provided.

4.1.1 Publication date

The publication dates of articles included in the database range from 1987 to 2013 (Figure 1); over 100 records were identified in each year from and including 2008 and reached over 140 articles in 2012. The 2013 publication year is not yet complete but already contains over 100 articles (as of the 14th October 2013 search date).

4.1.2 Overall relevance classification

255 (26 %) records were identified as having minor relevance to the project, reasons for this classification are documented for each record and included lack of key citation details, such as the abstract; records that represent a conference abstract rather than a published article or review; studies that dealt exclusively with the pure chemistry of PBDEs and determination of physicochemical properties; studies that dealt exclusively with the flame-retardant properties of PBDEs.

54 records corresponded to articles for which the full text was not in English. A full analysis of these papers is not possible. The overwhelming majority of these papers were in the Chinese language, as follows: Chinese (46 records), Polish (2), Japanese (4), German (2).

4.1.3 Further relevance classification

The 729 records deemed to be relevant were further classified. Regarding the human or environmental focus (records could be assigned to both categories): 45% (328/729) of records focused on human and 62% (449/729) focused on environmental data. Regarding relevance to major interest of the project 35% (255/729) had some relevance to the biotransformation of BDE-209; 75% (547/729) were relevant to likely BDE-209 and PBDE mixtures and 18% (130/729) had relevance to BDE-209 hazards and effects.

4.1.4 Organisms, cell lines studied

Across all the records, some 259 different entities (organisms, organism groups or cell lines) had been studied. Most (around 190 out of 259) of these entities were only examined in one of the studies. The most studied organism was human (116 records), who dominated the groups of mammals being studied. Rat and mouse were also frequently studied, with 17 studies (at abstract level) identifying the studied organism as 'rat' and 12 reporting studying the specific Sprague Dawley strain. Around 40 species or major groupings of birds were studied, with peregrine falcon (12) and herring gull(12) being frequently studied. More than 50 species of fish were studied, with carp (10) being most frequently studied. 13 species of plant were studied, including ryegrass (3 records).

4.1.5 Tissue sampled

In studies that reported sample collection the following tissues were reported: serum, blood (66); liver (36); eggs, usually bird (35); breast milk, usually human milk (31); muscle (28); adipose tissue or blubber (21), and 41 other sample types. Environmental samples included: air, soil, sediment, dust and sewage sludge.

4.1.6 Geographical regions

The geographical region being studied included 50 different countries (11 of which were only examined in a single study), 7 seas and 3 regions (Arctic, Antarctic, Great Lakes). The most frequently studied country was China (173 records), followed by the United States (60), Canada (32), Sweden (32) and Spain (26). The following countries were studied in between 10 and 20 records: Norway(16), Belgium(15), United Kingdom(15), Great Lakes(14), Japan(12), Australia(11), Germany(11), Italy(11), Taiwan(11), Korea(10).

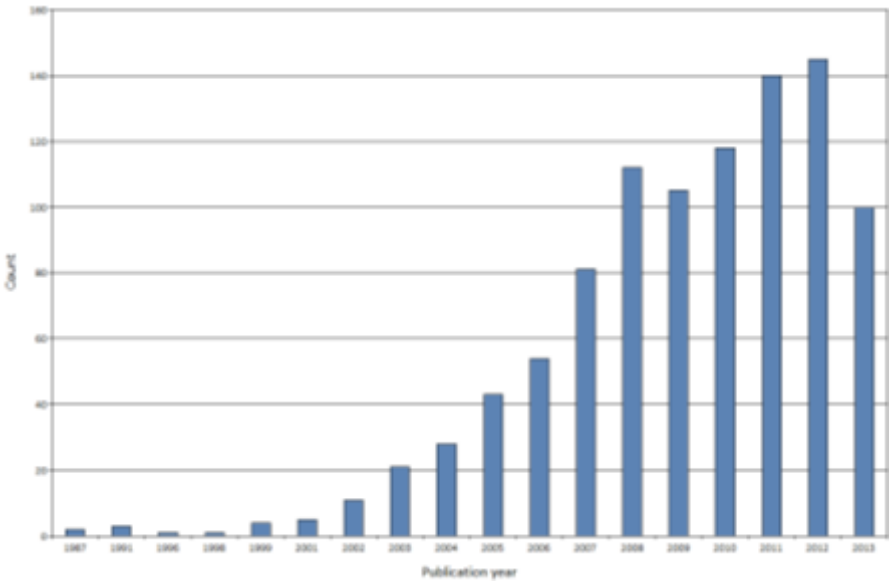
4.1.7 Toxic effects and target organs

Compilation of the effects, toxicities, modes and mechanism listed in abstracts produced over 80 items, however these items are defined by the individual authors of the scientific studies and do not constitute an organised classification. The common topics were neurotoxicity, oxidative stress, disrupted thyroid hormone homeostasis, affected development and behaviour, and immune system toxicity. Six target organs were studied: brain, fetus, kidney, liver, testes and thyroid; and the body systems examined were: nervous, immune, reproductive and endocrine(thyroid).

4.1.8 Chemical indexing

501 studies were suitable for indexing the PBDE congeners that they reported studying. Further analysis of the resulting data is provided in the relevant sections of the narrative review.

Figure 1: Publication year for articles included in the database



4.2 Results of grey literature search

In addition to the documents identified following the research strategy, a number of reports were provided by the Norwegian Environmental Agency and other documents were uncovered as the project progressed. A total of 59 documents were identified and are listed in Appendix B. A breakdown of the publishing organizations and the number of documents is given in Table 1.

Table 1: Breakdown of the source and number of official reports

Government reports	**27**
Australia	6
Canada	6
Japan	1
New Zealand	1
Norway	7
United Kingdom	2
United States	4
Multilateral organisations	**11**
Arctic Council	1
Norden	2
OSPAR	2
UNEP	6
European Union	**21**
European Commission	2
European Chemicals Bureau	6
ECHA	3
EFSA	1
JRC	9

The systematic search identified scientific literature and reports to serve as the basis for a narrative review (Section 5). To this end the PRISMA ("Preferred Reporting Items for Systematic Reviews and Meta-Analyses") checklist for reporting of narrative reviews (Moher et al. 2009) was completed and is included as Appendix C.

4.3 References

Moher D, Liberati A, et al. (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

UNEP. 2013, *Proposal to list decabromodiphenyl ether (commercial mixture, c-decaBDE) in Annexes A, B and/or C to the Stockholm Convention on Persistent Organic Pollutants,* UNEP/POPS/POPRC.9/2, Persistent Organic Pollutants Review Committee, Ninth meeting, Rome, 14–18 October 2013.

5. Narrative review

5.1 Human perspective

5.1.1 Biotransformation of BDE-209 in humans

5.1.1.1 Purpose of review

The purpose of this section is to review evidence of biotransformation of BDE-209 in test systems relevant to human toxicology.

5.1.1.2 Study selection

The literature describing biotransformation and metabolism of BDE-209 with relevance to human toxicology has been reviewed by EFSA (2011). That review was based on publications up to and including the year 2010. In this section, we briefly summarise the state of knowledge as described by EFSA (2011) and report on additional papers published after 2010.

Studies in rodents (oral dosing) and *in vitro* experiments with liver microsome preparations, usually also from rodents, have been utilised to assess of the potential of BDE-209 to undergo biotransformations in humans. For obvious reasons, direct evidence for BDE-209 biotransformations in humans with the chemically pure congener is not available, but the pattern of congeners observed in rodents after exposure to BDE-209 can be considered as informative for inferring biotransformation in humans. We therefore selected experimental studies with rodents and *in vitro* experiments with microsome preparations for review.

A factor that may complicate the interpretation of biotransformation studies is contamination of commercially available BDE-209 with other, lower brominated BDE congeners. If uncontrolled, claims of biotransformations of BDE-209, especially debromination reactions, may therefore be confounded by the presence of impurities. For this reason, emphasis was placed on studies that reported the purity and composition of BDE-209.

Another source of occurrence of lower brominated congeners from BDE-209 is photolytic degradation which can be controlled by shielding solutions from light. We therefore assessed whether protection from light sources was reported. A summary of all relevant studies can be found in Table 2.

5.1.1.3 Results

5.1.1.3.1 Early studies

Based on the studies by Morck et al. (2003), Sandholm et al. 2003, Huwe & Smith (2007) and Riu et al. (2008) which all utilised rats dosed orally with BDE-209, EFSA (2011) came to the conclusion that BDE-209 can undergo biotransformations to produce lower brominated congeners as well as hydroxylated and methoxylated transformation products.

5.1.1.3.2 Studies that identified specific BDE congeners

The standard of analytical methods available when the earlier studies by Moerck et al. (2003) and Sandholm et al. (2003) were conducted did not permit identification of specific congeners. For this reason, the transformation products observed could only be characterised in terms of number of bromine atoms. Studies carried out subsequently were able to identify specific congeners and demonstrated that BDE-209, given orally to rats, is debrominated to produce nona-BDEs (-206, -207, - 208), octa-BDEs (-196, -197/204, -198/203, -202)

and hepta-BDEs (-183). The nona-BDEs -206, -207 and -208 are by far the most abundant debromination products (Table 2).

Table 2: Studies of BDE-209 debromination

Author	Species, route of administration	Purity of BDE-209	Protection from photolytic decay	Metabolites observed (tissues in parentheses)	Most abundant metabolites, comments
Moerck 2003	Rat, oral	>98%	?	OH-penta, OH-hexa, OH-hepta BDE (liver)	Not quantified
Sandholm 2003	Rat (Sprague-Dawley), oral	>98%	?	Nona-BDE, OH-nona, OH-octa, MeO-octa (plasma)	Not quantified
Huwe and Smith 2007	Rat, oral	>98%	?	Nona: BDE-207 Octa: BDE-197, -201	BDE-207
Riu 2008	Rat (Charles-River), oral	>99.8%	?	Nona: BDE-206, 207, 208 (multiple tissues) Octa-BDE, OH-octa (multiple tissues)	BDE-207, > -206, -208
Cai 2011	Rat (Sprague-Dawley), oral	>98%, impurities: BDE-208, -207, -206, octa	yes	Nona: BDE-206, -207, -208 (blood and placenta of dams, fetus) Octa: BDE-196, -197/204, -198/203 (blood and placenta of dams, fetus)	BDE-207 > -206 ~ -208 Hydroxylated metabolites not reported or detected
Wang 2011	Rat (Sprague-Dawley), oral	>99%	?	Nona: BDE-206, -207, -208 (kidney, liver, adipose) Octa: BDE-196, -197, -202, -203 (kidney, liver) Hepta: BDE-183 (adipose)	BDE-207, > -206 (liver, adipose) BDE-197 (kidney) Hydroxylated metabolites not reported or detected
Zhang 2011	Rat (Sprague-Dawley), oral	>98% impurities: BDE-208, -207, -206, octa	yes	Nona: BDE-206, -207, -208 (pup liver, kidney, brain) Octa: BDE-196, -197/204, -198/203 (pup liver, kidney, brain)	BDE-207, ~ -206 (pup liver, kidney, brain) Hydroxylated metabolites not reported or detected
McKinney 2011	Rat liver microsomes (Wistar Han rats)	?	?	No metabolites detected	

5.1.1.3.3 Indirect evidence for biotransformation reactions of BDE-209

Indirect evidence for the reality of debromination reactions can be obtained from *in vitro* studies with preparations of hepatic microsomes (McKinney et al. 2011). McKinney et al. (2011) studied biotransformations of BDE-209 by using rat liver microsomes and observed substantial loss of the parent compound (14-25%). However, despite this depletion of the parent BDE-209 in their preparations, they were unable to detect any transformation products. McKinney et al. offer several explanations for this observation, including problems with complete derivatisation of debromination products, low recovery of unknown major metabolites and covalent binding of metabolites to macromolecules. Substantial fractions of non-extractable metabolites have been noted in other studies (Rui et al. 2008) and the majority of the original doses of BDE-209 given to rats could not be accounted for in terms of the measured metabolite concentrations in tissues, also suggesting that significant fractions of conversion products were not extracted (Moerck et al. 2003, Sandholm et al. 2003, Huwe and Smith 2007).

5.1.1.3.4 Impurities of BDE-209 as a confounding factor

Some of these debromination products occur as impurities of the commercially available BDE-209 used for dosing in these studies. As pointed out by Biesemeier et al. (2012), this makes it difficult to ascertain that congeners detected in tissues have in fact arisen from debromination reactions and are not simply the result of impurities. As shown in Table 2, this is a fundamental difficulty affecting all experimental studies with BDE-209, since it is technically not possible to synthesise BDE-209 totally free of lower brominated congeners. BDE-209 of a purity >98% was used by most authors. To distinguish debromination by metabolism from the occurrence of impurities, it is necessary to analyse impurities in the test substance for any overlaps with putative metabolites.

Cai et al. (2012) reported the impurities of the BDE-209 used in their studies (Cai et al. 2011, Zhang et al. 2011) as BDE-206 (0.3%), -207 (0.7%) and -208 (0.4%), with a non-quantifiable trace of octa-BDE. Although all these congeners occurred in rat tissues after exposure to BDE-209, the observation of additional debrominated congeners, including BDE-196, -197/204, 198/203 cannot be explained without invoking debromination *in vivo*, and is good evidence that debromination has indeed occurred.

5.1.1.3.5 Hydroxylation reactions

The earlier rat dosing studies (Moerck et al. 2003, Sandholm et al. 2003, Riu et al. 2008) reported several debrominated hydroxylated and methoxylated metabolites, including OH-hexa-BDE, OH-hepta BDE, OH-octa-BDE, MeO-octa-BDE and OH-nona-BDE. The occurrence of hydroxylated or methoxylated metabolites has not been reported in the more recent BDE-209 dosing studies (Cai et al. 2011, Wang et al. 2011, Zhang et al. 2011).

The data reported by Moerck et al. (2003), Sandholm et al. (2003) and Riu et al. (2008) suggest that reductive debromination of BDE-209 to nona-, octa- and hepta BDEs is the first step in its biotransformation. The debrominated conversion products then undergo oxidation reactions to form hydroxylated and methoxylated products. These oxidation products could be substrates for conjugation reactions to yield water-soluble metabolites which can be excreted via the bile, urine and faeces (EFSA 2011).

It is unclear which enzymes are involved in the debromination reactions, but it has been suggested that iodothyronine deiodinases are likely candidates (Wang et al. 2010). The oxidation reactions are likely to be carried out by as yet unidentified CYP isoforms (EFSA 2011).

5.1.1.3.6 Conclusions

There is good evidence from oral dosing studies in rats for biotransformation of BDE-209 to lower brominated congeners. Nona-BDEs (-206, -207) are the most abundant conversion products, but octa-BDEs (-196, -197/204, -198/203, -202) and hepta-BDEs (-183) have also been identified. These lower brominated congeners can undergo hydroxylation reactions to yield hydroxylated and methoxylated conversion products, mostly derived from nona-, octa- and hepta BDEs. These oxidated metabolites are the substrates for conjugation reactions (phase II metabolism).

5.1.2 Likely human exposure to PBDE mixtures, including after biotransformation

5.1.2.1 Purpose of review

The section on biotransformation reactions of BDE-209 in test systems relevant to mammalian toxicology (5.1.1) established that this congener can be debrominated to nona-, octa- and hepta-BDE. Nona-BDEs (-206, -207) are the most abundant conversion products, with evidence of further debromination to octa-BDEs (-196, -197/204, -198/203, -202) and hepta-BDEs (-183). Lower brominated congeners can undergo hydroxylation reactions to yield hydroxylated and methoxylated conversion products, mostly derived from nona-, octa- and hepta BDEs.

The purpose of this section is to identify relevant combinations of PBDEs to which humans are exposed. Special attention is paid to the occurrence of BDE-209 biotransformation products in human tissues, body fluids, food items and other media relevant to human exposures.

5.1.2.2 Study selection

Human exposures to BDEs have been reviewed by Frederiksen et al. (2009), EFSA (2011) and Domingo (2012). These reviews reveal a lack of uniformity in terms of the BDE congeners that were measured and reported. What is reported as «sum PBDE» depends on the congeners selected for measurement, and comparability between studies is problematic when different congeners have been summed up. Especially the older literature did not include BDE-209, and while this congener is now more regularly measured, even recent studies, particularly from the USA, often do not consider BDE-209, despite the fact that it constitutes a large fraction of the total.

There is no consensus on which BDE congeners to measure in human samples, food samples, air or dust, presumably because of a lack of agreement on which congeners should be regarded as toxic congeners of concern. Based on their occurrence in the environment and in food, EFSA (2011) considered the following eight BDE congeners as being of primary interest for human exposures: BDE-28, -47, -99, -100, -153,-154, -183 and -209. These eight congeners occur together in a wide variety of environmental media and food items, and accordingly, they are the focus of this review. Literature that did not contain data about BDE-209 was excluded from consideration. Occasionally, other congeners have also been reported, including BDE-17, -66, and -85, but the measured levels were generally very low. For this reason, they are not considered here.

In view of their relevance as BDE-209 debromination products we chose studies that in addition to BDE-28, -47, -99, -100, -153,-154, -183 and -209 also identified at least one of the following congeners: BDE-196, -197/204, -198/203, -202, -206 or -207. Studies that did not contain data about at least one of these congeners were generally not considered.

Studies that did not contain congener-specific data (i.e. literature that reported only summed values “Sum PBDE” or “octa-BDE” etc.) were excluded.

Since tetra- to octa-BDE have the highest stability and persistence of all PBDEs (EFSA 2011), they are prone to bioaccumulation in human tissues. The bioaccumulative properties of BDE-209 appear to be species-dependent, with evidence that they are capable of bioaccumulation in human tissues (Frederiksen et al 2009). For this reason, levels measured in human adipose tissue or the lipid fraction of breast milk, serum, blood and placenta can give a reflection of internal exposures received through all relevant routes, including food and inhalation of dust. We therefore focused on literature that determined PBDEs in these tissues and body fluids. Since PBDE levels in breast milk or colostrum are of direct relevance to determining the exposure to nursing infants, emphasis was placed on locating literature describing PBDE levels in these media.

There is evidence of considerable binding of BDE-209 to proteins (Hakk et al. 2002) which means that analyses of levels in lipids may underestimate the body burden of BDE-209 and other BDE congeners. However, tissues or body fluids representative of protein-bound fractions of BDE-209 and other BDEs have not been the focus for human biomonitoring studies.

We identified a few studies where PBDE levels in hair were determined, however, these studies were excluded from further consideration of human internal exposures because of difficulties in distinguishing deposition onto hair via external sources (dust, hair care products contaminated with PBDEs) from incorporation into hair via internalised BDE's. We also excluded studies measuring PBDE levels in semen, because the relationship of concentrations in semen with those in serum and adipose tissue is not well defined.

PBDE levels in human tissues, food items, air and dust are reported as means, including the range. The distributions of PBDEs in human tissues and environmental samples are typically quite skewed, with sometimes very high concentrations at the upper bound. In such cases, standard deviations are not meaningful statistics and were therefore not reported. Some authors provided medians instead of means, and when this was the case, values were reported as provided by the authors.

During the compilation of relevant data it became obvious that there is no consistency in the way in which non-detects are reported. Sometimes, the limit of quantitation (LOQ) was chosen, sometimes 50% of the LOQ. Frequently, non-detects were simply reported as "0". Uniformity in documenting literature data is further complicated by the omission of details of the LOQ and the limits of detection (LOD) in many studies. For the sake of simplicity, we report non-detects as provided by the authors of the relevant studies. When possible, values below the LOQ are shown in italics.

In summary, studies which met the following criteria were analysed:

- Contains data on BDE-209 AND at least one of the congeners BDE-196, -197/204, -198/203, -202, -206 or -207
- Analyses human blood, breast milk, colostrum, cord blood, placenta and serum or plasma
- Analyses congener-specific BDE concentrations in the exposure media food, drinking water, surface soil, indoor dust, outdoor air, indoor air

5.1.2.3 Results

5.1.2.3.1 The occurrence of BDE-209 biotransformation products in human tissues – evidence for transformation reactions in humans

The systematic search identified a total of 55 studies which measured BDE-209 in human blood, breast milk, colostrum, cord blood, placenta, semen and serum. Of these, only 19 also provided data on at least one of the BDE-209 biotransformation products shown to be formed in the wake of controlled exposures of mammalian test organisms to BDE-209. These 19 studies therefore met the selection criteria.

By far the most comprehensive analysis of BDE congeners, including octa- and nona-BDE's, has been conducted by (Antignac et al. 2009). In addition to the most frequently occurring tetra-, penta- and hexa-BDEs, they were able to detect known debromination products of BDE-209 (BDE-196, -197, -201, -202, -203, -206, -207, -208) in breast milk from French women. BDE-196, -197, -203, -206, -207, -208 were also found in breast milk from Taiwan (Koh et al. 2010) and China (Ma et al. 2012). BDE-197, -203, and -207 were identified in breast milk from Sweden (Jakobsson et al. 2012), but at quite low levels (below the level of quantitation, but not below the detection limit).

Several BDE-209 debromination products were detected in serum (BDE-197, -203, -207, Jakobsson et al. 2012; BDE-183, -197, Lunder et al. 2010; -183, -197, -207, Eguchi et al. 2012; -183, -197, -203, -207, Zhu et al. 2009), whole blood (-183, -196, -197, -207, Uemura et al. 2010; -183, -197, Zhao et al. 2013), cord blood and serum (BDE-197, -203, -207, Jakobsson et al. 2012; -183, -196, -197, Gomara et al. 2007), placenta (BDE-196, -197, Gomara et al. 2007) and aborted foetuses (BDE-197, Zhao et al. 2013).

A study of human serum samples from residents of an electronic waste dismantling site in Guiyu town, South China provided evidence of hydroxylated PBDEs. Three OH-PBDEs, including two hydroxylated octabromodiphenyl ethers (OH-octaBDEs, 6-OH-BDE-196 and 6-OH-BDE-199) and one hydroxylated nonabromodiphenyl ether (OH-nonaBDE, 6′-OH-BDE206) could be detected in pooled serum samples, showing that debromination products derived from BDE-209 can be metabolised oxidatively in humans (Yu et al. 2010).

In conclusion, data from human biomonitoring studies conducted independently in several countries from different continents provide good evidence that BDE-209 debromination and oxidation reactions first identified in experimental studies with rodents also occur in humans. These are the result of debromination reactions, as well as direct exposure to nona- and octa-BDE through commercial mixtures. In addition to these higher brominated congeners, humans can be exposed to congeners with even fewer bromine atoms that originate from BDE-209 through abiotic and biotic transformation (see Section 5.2.1).

5.1.2.3.2 Human exposures to PBDE combinations

Human populations worldwide are exposed to combinations of PBDEs. Exposure can occur in foetal life through trans-placental transfer from the mother, in neonatal life through breast feeding, and during later life stages via food and ingestion of dust. It has become clear that both the composition of PBDE combinations relevant to human exposures, and the extent of exposure to specific congeners in life stage dependent. For this reason, we discuss relevant life stages separately, including foetal and neonatal life, infants up to the age of 6 months, toddlers and young children up to age 3 years and adult life.

5.1.2.3.3 Foetal and neonatal exposure

Four studies have measured PBDEs in foetal tissues and have provided direct evidence that PBDEs can cross the placenta and reach the foetus (Schecter et al. 2007, Doucet et al. 2009, Rawn et al. 2011, Zhao et al. 2013). Of these, Schecter et al. (2007), Rawn et al. (2011) and Zhao et al. (2013) analysed BDE-209, but only Zhao et al. (2013) met the selection criteria of this report by determining BDE-209 levels and at least one of its debromination products, BDE-197. The BDE-209 levels in the foetal livers analysed by Schecter et al. (2007) were found to be below the limit of detection.

The study by Zhao et al. (2013) provides details of the placental transfer of PBDEs to the foetus. Paired aborted foetuses from 10-13 weeks of gestation (n=65), placentas (n=65) and maternal blood samples (n=31) were analysed for BDE-28, -47, -99, -100, -153, -154, -183, -197 and -209. While the congener spectrum was very similar in the foetuses, placentas and maternal blood, the PBDE levels were generally highest in maternal blood, on a ng/g lipid basis. By analysing paired samples, Zhao and colleagues were able to establish that the lower brominated BDE-28, -47 and -99 present in maternal blood cross the placenta more easily to reach the foetus than the higher brominated BDE-153, -197 and -209.

The details of the processes that determine BDE transfer from the mother to the foetus remain to be worked out, but differences in the toxicokinetic features of the congeners are likely play a role. For the low- to moderately brominated BDE (tri- to hepta-) with their long half-lives of elimination (up to several years, Jakobsson et al. 2012) a steady state will have established itself in maternal serum. In contrast, highly brominated BDE's (octa- to deca-) have shorter half-lives of elimination (in the range of several weeks), and this may well influence the differential transport processes observed by Zhao et al. (2013).

The two studies of PBDE levels in placenta samples that met the inclusion criteria of this review are those by Gomara et al. (2007) and Zhao et al. (2013).

On the basis of the data provided by Zhao et al. (2013) it is possible to estimate the body burden of foetuses at 10 – 13 weeks of gestation for each measured congener, by using the mean lipid content of 1.07% given by the authors, with the proviso that the true body burden may be higher, due to protein binding of BDE-209 and other congeners which cannot be captured by analysing BDE levels in lipids. From the given mean BDE concentrations, the resulting body burdens for BDE-28, -47, -99, -100, -153, -154, -183, -197 and -209 are 3.8, 6.2, 2, 0.7, 6.2, 0.5, 2, 7.7 and 15.5 ng/kg body weight, respectively.

PBDE levels in cord blood serum give indications of the body burden of neonates. We located two studies of PBDE levels in cord blood serum that met our selection criteria (Jakobsson et al. 2012, Gomara et al. 2007), Table 3. The Swedish study (Jakobsson et al. 2012) showed lower levels than the Spanish study (Gomara et al. 2007), but there were similarities in the congener patterns, with BDE-47 and -209 making a large contribution to the sum of PBDE. In the Spanish samples, BDE-47, -99, -100 and -209 contributed most. The levels of BDE-28, -154 and -197 were relatively low in both cohorts, with values often below the limit of quantitation.

Table 3: PBDE congeners in cord blood samples (ng/g lipid weight)

italics: below LOQ

Country	Sampling	N	Statistic	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-196	BDE-197	BDE-201	BDE-202	BDE-203	BDE-206	BDE-207	BDE-208	BDE-209	Sum
Sweden	2005-2006	9	median	*0.04*	1.65	0.12	*0.26*	0.84	*0.08*			0.37			*1.12*		*0.75*		4.70	9.93
Jakobsson 2012			min	*0.04*	0.58	0.12	*0.26*	0.15	*0.08*			0.37			*0.37*		*0.75*		1.54	
			max	*0.29*	5.83	0.37	*0.79*	1.29	*0.24*			1.12			*1.60*		*2.29*		9.50	
	Congener profile		%	0.004	0.166	0.012	0.026	0.084	0.008			0.037			0.113		0.075		0.473	
Spain Vallecas Gomara 2007	2003-2004	44	median	*0.080*	3.30	4.30	2.30	0.52	0.13	1.30	0.03	0.300							2.20	14.46
			min		0.03	1.80	1.00	0.18	0.10	0.06	0.02	0.100							1.10	
			max		35.00	17.00	5.70	4.40	1.60	6.00	3.00	3.500							11.00	
	Congener profile		%	0.006	0.228	0.297	0.159	0.036	0.009	0.090	0.002	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.152	
Spain Getale Gomara 2007	2003-2004	48	median	*0.060*	3.20	3.00	1.50	0.32	*0.10*	0.15	0.02	0.100							1.40	9.85
			min		0.03	0.94	0.81	0.18		0.06	0.02	0.100							1.10	
			max		10.00	7.40	4.40	8.70		2.90	1.80	1.500							24.00	
	Congener profile		%	0.006	0.325	0.305	0.152	0.032	0.010	0.015	0.002	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.142	

5.1.2.3.4 Exposure of breast-fed neonates and infants up to 3 months of age

The PBDE exposure of breast-fed neonates and infants up to the age of 3 months is strongly determined by PBDE levels in colostrum and mature breast milk.

There are significant changes in the composition of breast milk during the period of lactation and these changes have been shown to impact on the BDE congener profile. Colostrum is secreted during the first five days after birth and is rich in immunoglobulin A, lactoferrin and vitamin A, but relatively low in fat and lactose compared to mature breast milk. The process of breast milk maturation takes about 14 days (see the literature cited in Jakobsson et al. 2012).

Jakobsson et al. (2012) have shown that colostrum is enriched in higher brominated BDE, including BDE-197, -207 and -209, relative to mature breast milk. Depending on the congener, the levels in colostrum were by a factor of 3 (BDE-207), 7 (BDE-209) or 9 (BDE-197) higher than in mature breast milk. This means that exposure estimates for neonates based on the congener levels in mature breast milk will underestimate the intake of neonates. Unfortunately, the measurement of BDEs in colostrum is not commonly undertaken, and the study by Jakobsson is the only one that met the inclusion criteria of this project.

In accordance with the selection criteria for this report, we located the following studies of PBDE levels in breast milk, here arranged according to geographical regions: Europe – Antignac et al. (2009), Gomara et al. (2007), Jakobsson et al. (2012); Africa (Ghana): Asante et al. (2011); Asia (India): Devananthan et al. (2012); East Asia (China, Taiwan, Vietnam): Chao et al. (2011), Koh et al. (2010), Ma et al. (2012), Sudaryanto et al. (2008), Tue et al. (2010); East Asia (Philippines): Malarvannan et al. (2013)(2009). Most studies collected breast milk samples from the general population. In one study (Malarvannan et al. 2013), samples from mothers living near a waste dump site in the Philippines were taken and compared to a reference group. No differences were observed.

The congener levels determined in breast milk vary considerably within and between geographical regions. However, a feature common to all studies are the high levels reported for BDE-47, -99, -153 and -209. Relatively low were BDE-154, -183, -197, -203 and -207, although exceptions were notable in the Philippines (comparatively high levels of BDE-183, Malarvannan et al. 2009) and in China (comparatively high levels of BDE-197, Sudaryanto et al. 2008, Ma et al. 2012).

Table 4 gives the ranges of mean daily intake values for breastfed infants across various regions in Europe, Africa and Asia which were derived from the literature listed above. In estimating these intakes, it was assumed that an infant weighing 6.1 kg consumes 800 ml (average consumption) or 1200 ml (high consumption) of breast milk with a fat content of 3.5% every day (EFSA 2011). Due to the scarcity of analyses of colostrum, it was not possible to assess the true exposure to BDE-209 and other higher brominated congeners in the first few weeks of a babies' life which derives from the enrichment of BDE-197, -207 and -209 in colostrum (Jakobsson et al. 2012).

Table 4: Mean BDE intakes of breastfed infants across regions (ng/kg d)

LB: lower bound

UB: upper bound

based on average breast milk consumption of 800 ml per day

	Europe		Africa (Ghana)	Asia (India)	Asia (China, Taiwan)		Asia (Philippines, Vietnam)	
Congener	LB	UB			LB	UB	LB	UB
-28	0.05	0.83	0.32	0.29	0.43	2.1	0.13	1.38
-47	0.74	16	7.5	2.1	2	2.7	0.6	16.1
-99	1.75	5	2.3	0.21	0.55	0.87	0.26	5.1
-100	1.9	3	1.3	0.51	0.64	0.85	0.18	3.1
-153	0.46	8.65	1.1	1.1	4.4	3.6	0.45	2.57
-154	0.05	1.46	0.12	0.42	0.32	0.4	0.09	0.6
-183	0.78	1.38	0.51	0.33	0.71	1.2	0.13	6.9
-196	0.23	0.55	0.18	0.1	0.14	2.12	0.05	0.09
-197	0.37	2.16	0.92	0.38	0.98	6.72	0.21	1.01
-201	0.56							
-202	0.46							
-203	0.55				0.37	2.12		
-206	2.53		0.18	0.33	0.29	1.38	0.28	
-207	0.48	3.95	0.42	0.83	0.69	5.2	0.11	0.92
-208	1.15				0.29	1.98		
-209	1.01	13.3	4.6	3.82	2.2	13.8	2.3	7.8

based on high breast milk consumption of 1200 ml per day

	Europe		Africa (Ghana)	Asia (India)	Asia (China, Taiwan)		Asia (Philippines, Vietnam)	
Congener	LB	UB			LB	UB	LB	UB
-28	0.08	1.25	0.48	0.44	0.65	3.15	0.20	2.07
-47	1.11	24.00	11.25	3.15	3.00	4.05	0.90	24.15
-99	2.63	7.50	3.45	0.32	0.83	1.31	0.39	7.65
-100	2.85	4.50	1.95	0.77	0.96	1.28	0.27	4.65
-153	0.69	12.98	1.65	1.65	6.60	5.40	0.68	3.86
-154	0.08	2.19	0.18	0.63	0.48	0.60	0.14	0.90
-183	1.17	2.07	0.77	0.50	1.07	1.80	0.20	10.35
-196	0.35	0.83	0.27	0.15	0.21	3.18	0.08	0.14
-197	0.56	3.24	1.38	0.57	1.47	10.08	0.32	1.52
-201	0.84							
-202	0.69							
-203	0.83				0.56	3.18		
-206	3.80		0.27	0.50	0.44	2.07	0.42	
-207	0.72	5.93	0.63	1.25	1.04	7.80	0.17	1.38
-208	1.73				0.44	2.97		
-209	1.52	19.95	6.90	5.73	3.30	20.70	3.45	11.70

5.1.2.3.5 Infants and children 6 month to 3 years old

Several studies established that toddlers and young children show higher levels of PBDEs than adults. Lunden et al. (2010) assessed PBDEs in blood samples collected concurrently from 20 mothers and their children, ages 1.5 to 4 years. The sum of PBDEs in children's blood was 2.8 times higher than in mothers, when compared on a lipid basis, and this was also the case for BDE-209 and -197. Similar results were obtained by Toms et al. (2008, 2009) in samples from Australia, although BDE-209 or any of its higher brominated biotransformation products were not measured in these studies. Toms and colleagues found that the PBDE concentrations peaked in the age group of 3 years. It is hard to explain this peak in terms of transfer of PBDEs via breast milk, since breast feeding normally ceases well before age 3 years. Toms et al. suggested that young children either are especially highly exposed to PBDEs and/or have a diminished capacity for elimination.

While there is no evidence for a diminished capacity of young children and toddlers to eliminate PBDEs, it appears that children receive considerable PBDE exposures via house dust, as a result of their play behaviour. Lorber (2008) estimated that the total PBDE exposure of children of age 1-5 years is 7-fold higher than for adults, on a body weight basis. He attributed this to higher dust ingestion by children and concluded that the pathways of soil/dust ingestion and dermal contact overwhelm the exposure of children (and adults) to PBDEs. BDE-209 alone contributed more than half of the total PBDE exposure. Webster et al. (2005) estimate that more than half of a child's PBDE exposure via dust is dermal.

EFSA (2011) reviewed the occurrence of PBDE in air and dust. The concentrations in outdoor air and dust are considerably lower than those in indoor air and dust and have been judged to be of no major importance in contributing to the exposure of young children.

In contrast, indoor dust is a major source of exposure, contributing up to 60% of the total exposure of young children (Harrad and Abdallah 2011). The congener profile in household dust and car dust is dominated by BDE-209 and its debromination products. Median BDE-209 levels in car cabin dust are 190,000 ng/g (Harrad and Abdullah 2011). In house dust from Europe (Germany, Sweden, UK), BDE-209 concentrations ranged from 63 – 10,000 ng/g. In contrast, the concentrations of BDE-47, -99, -100, -153, -154 and -183 taken together were between 26 and 170 ng/g (Fromme et al. 2009).

While the studies authored by Harrad and Abdullah (2011) and Fromme et al. (2009) did not include known BDE-209 debromination products, more recent studies measured a wider range of congeners, including BDE-196, -197, -206, -207 and -208. These more recent studies confirmed that BDE-209 is the prominent congener in indoor dust, but highlighted that sometimes BDE-206 and -207 also contribute strongly. A compilation of recent measurements of PBDE in indoor dust from houses, offices and cars which also measured BDE-209 debromination products is presented in Table 5.

Based on a comprehensive survey of 3933 samples of food items across European countries compiled by EFSA (2011), it appears that the PBDE exposures from food for the age classes 6 months to 1 year, and 1-3 years is also higher than for all other age groups. This European food survey did not consider BDE-196, -197, -206, -207 and -208 and focused instead on the eight congeners classed by EFSA as relevant (BDE-28, -47, -99, -100, -153,-154, -183 and -209). Apart from sporadic measurements in isolated food items, data of comparable quality for BDE-196, -197, -206, -207 and -208 are not available. For this age group it is therefore not possible to derive exposure estimates for all routes that include BDE-196, -197, -206, -207 and -208.

The estimated combined intakes for dust and food in the summary Table 6 represent upper bounds. The data show that children experience the highest PBDE intakes of all age groups, on a body weight basis. BDE-47, -99 and -209 make the highest contribution to the total intake.

The dust intakes were estimated on the basis of the data compiled in Table 5, assuming a dust ingestion rate of 50 – 200 mg/d (Wilford et al. 2005) and a body weight of 15 kg. It is apparent from Table 6 that ingestion of dust can contribute more than 50% of a child's total PBDE exposure. The compilation in Table 5 is based on the work by Wei et al. (2009), Toms et al. (2009), Watkins et al. (2013), Thuresson et al. (2012) and Kang et al. (2011).

Table 5: PBDE congeners in dust samples (ng/g)

italics: below LOQ

Country	N	Statistic	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-196	BDE-197	BDE-201	BDE-206	BDE-207	BDE-208	BDE-209	Dust source
USA	7	mean	6.1	571.1	842.7	136.0	58.7	49.6	10.9	3.0			65.4	45.7		1275.0	house
		min	0.5	22.3	43.0	9.0	4.0	3.0	4.0	2.0			15.6	15.0		360.0	
Wei 2009		max	11.5	2075.0	2924.0	464.0	141.0	117.0	27.0	5.0			104.0	93.0		4156.0	
USA	2	mean	3.5	370	490	82	33.5	28.5	3.75	4.15			178	92.5		2721.5	car
		min	2.5	344	448	76	27	23	3.5	4			172	87		2621	
Wei 2009		max	4.5	396	532	88	40	34	4	4.3			184	98		2822	
Australia	10	mean		91.6	184.6	37.5	23.8		102.4							377.5	house
		min		24.0	36.0	10.0	1.0		0.0							95.0	
Toms 2009		max		434.0	862.0	155.0	139.0		948.0							1585.0	
USA	31	geo mean	7.5	697.0	915.0	195.0	138.0	115.0	81.0	29.0	32.0	4.9	153.0	125.0	62.0	4204.0	office
		min	0.4	37.0	0.4	13.0	11.0	8.0	15.0	7.0	4.0	1.0	29.0	22.0	10.0	912.0	
Watkins 2013		max	207	19494.0	32831.0	8672.0	5973.0	5202.0	12970.0	2858.0	6109.0	359.0	3395.0	4312.0	1710.0	106204.0	
Sweden	10	median	1.3	42.0	52.0		6.6		12.0		5.1		0.0	10.0	3.0	320.0	house
		min	0.1	0.5	1.0		0.6		0.7		1.0		30.0	2.0	3.0	51.0	
Turesson 2012		max	5.6	230.0	140.0		23.0		49.0		40.0		140.0	96.0	52.0	3600.0	
Sweden	34	median	0.8	37.0	66.0		7.8		11.0		1.4		0.0	20.0	0.0	1100.0	home
		min	0.1	0.5	1.0		0.2		0.7		1.0		30.0	2.0	3.0	50.0	
Turesson 2012		max	9.2	280.0	1200.0		410.0		110.0		45.0		390.0	1000.0	430.0	100000.0	

Table 5 cont.: PBDE congeners in dust samples (ng/g)

italics: below LOQ

Country	N	Statistic	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-196	BDE-197	BDE-201	BDE-206	BDE-207	BDE-208	BDE-209	Dust source
Sweden	10	median	1.2	52.0	92.0		23.0		55.0		29.0		0.0	44.0	17.0	780.0	home
		min	0.1	14.0	14.0		4.3		15.0		7.0		30.0	20.0	10.0	540.0	
Turesson 2012		max	5.4	390.0	770.0		100.0		160.0		100.0		340.0	160.0	96.0	12000.0	
Sweden	10	median	2.8	120.0	110.0		12.0		6.5		4.2		0.0	15.0	8.0	580.0	Day care centres
		min	0.1	31.0	42.0		6.0		2.7		1.7		30.0	7.0	4.0	180.0	
Turesson 2012		max	8.2	910.0	550.0		19.0		15.0		6.5		110.0	58.0	29.0	3500.0	
Sweden	4	median	0.2	7.4	11.0		3.0		2.2		2.7		19.0	17.0	12.0	1300.0	Car
		min	0.1	0.6	1.5		0.3		0.7		1.0		30.0	2.4	3.0	50.0	
Thuresson 2012		max	0.4	22.0	30.0		7.0		6.7		18.0		1700.0	540.0	110.0	28000.0	
China	55	median	23	109.0	186.0	144	25.0	18.0	28.0	7.4	9.5		65.0	62.0		1401.0	work
		min	1.7	2.0	6.0	10	0.0	0.0	2.6	1.0	0.0		10.0	9.0		103.0	
Kang 2011		max	100	1586.0	10100.0	458	908.0	876.0	133.0	133.0	96.0		463.0	455.0		37440.0	
China	23	median	37	102.0	75.0	85	11.0	8.4	78.0	12.0	15.0		50.0	47.0		975.0	house
		min	7	27.0	15.0	22	0.0	0.0	14.0	1.5	2.3		13.0	13.0		346.0	
Kang 2011		max	122	2740.0	9447.0	221	650.0	714.0	797.0	36.0	277.0		564.0	635.0		15795.0	

Table 6: Estimated total PBDE intakes for different age groups (ng/kg d)

Upper bounds

Age class	Route	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-196	BDE-197	BDE-206	BDE-207	BDE-208	BDE-209	Total
Children	Food (EFSA 2011)	0.87	6.4	2.99	1.86	1.62	1.81	1.56						9.69	26.8
0.5-3 years	Dust	0.49	9.29	12.20	2.60	1.84	1.53	1.36	0.39	0.43	2.37	1.67	0.83	56.04	91.0
	Total	**1.36**	**15.69**	**15.19**	**4.46**	**3.46**	**3.34**	**2.92**	**0.39**	**0.43**	**2.37**	**1.67**	**0.83**	**65.73**	**117.8**
Adults	Food (EFSA 2011)	0.28	1.91	0.65	0.7	0.42	0.51	0.36						2.82	7.7
	Dust	0.05	1.00	1.31	0.28	0.20	0.16	0.15	0.04	0.05	0.25	0.18	0.09	6.01	9.8
	Total	**0.33**	**2.91**	**1.96**	**0.98**	**0.62**	**0.67**	**0.51**	**0.04**	**0.05**	**0.25**	**0.18**	**0.09**	**8.83**	**17.4**
Adults	USA	**0.029**	**1.971**	**2.186**	**0.829**	**0.229**	**0.150**	**0.064**						**2.114**	**7.6**
total	Lorber 2008														
Adults	Food (EFSA 2011)	0.28	1.91	0.65	0.7	0.42	0.51	0.36						2.82	7.7
	additional fish intake	0.23	5.36	0.75	2.07	0.47	0.59	0.58			0.08	0.15	0.05	1.77	12.1
	Dust	0.05	1.00	1.31	0.28	0.20	0.16	0.15	0.04	0.05	0.25	0.18	0.09	6.01	9.8
	Total	**0.56**	**8.27**	**2.71**	**3.05**	**1.09**	**1.26**	**1.09**	**0.04**	**0.05**	**0.34**	**0.33**	**0.14**	**10.60**	**29.5**

5.1.2.3.6 Exposure of adults

Similar to infants and children of 6 months to 3 years, relevant exposure routes for adults are ingestion of food and indoor house dust.

The values estimated for adults and shown in Table 6 are based on the comprehensive food survey in EFSA (2011). As for children, data that could form the basis for estimates for intake from food of congeners other than the eight PBDEs covered in EFSA (2011) were not available.

The estimated intakes from the exposure medium dust were calculated assuming a dust ingestion rate of 0.5 – 100 mg/d, and a body weight of 70 kg. These estimates show that dust can contribute substantially to the total PBDE exposure not only of children, but also of adults. The values in Table 6 agree fairly well with the estimates published by Lorber (2008) for adults in the USA.

High consumption of fish is considered as a diet of specific concern for PBDE exposure (EFSA 2011). This is true especially for people who eat fish every day, as is the case with fishermen or fish sellers or communities living in special regions of the world. For these frequent and high consumers of fish, a daily fish consumption of 2.6 g/kg body weight was retrieved from the Comprehensive European Food Consumption Database (for further details see EFSA 2011). The additional fish intake estimated for these population groups (Table 6) were taken from EFSA (2011). Estimates for the intake of BDE-206, -207 and -208 via fish were taken from sporadic reports of levels measured in fish (Carlsson et al. 2011, Chen et al. 2013, Qin et al. 2009, Zhang et al. 2010). As shown in Table 6, the PBDE intake for adults from this source can reach 40% of the total exposure.

5.1.2.3.7 Conclusions

There is good evidence that humans have the capacity to transform BDE-209 to lower brominated and hydroxylated products. These have been detected in human tissues. There are both abiotic and biotic processes in the environment and in other organisms that lead to the liberation of additional transformation products with lower degrees of bromination. Humans are exposed to these transformation products through the ingestion of contaminated food or dust.

Human PBDE exposures depend strongly on the life stage. On an amount per body weight basis, children of age 1 – 3 years are the age group with the highest PBDE exposures. Breastfed infants are also quite highly exposed. With increasing age, the intake (on a body weight basis) declines somewhat.

For all age groups and life stages, BDE-47, -99 and -209 contribute most to the overall total exposure, from all routes. BDE-209 alone makes up 50% or more of the total PBDE exposure.

5.1.3 Possible toxic effects of PBDE mixtures in human

5.1.3.1 Purpose of review

The preceding sections have identified the PBDE congeners that constitute human relevant combined exposure scenarios, for a variety of exposure media. The purpose of this section is to consider the toxicity of these congeners to humans, with an emphasis on BDE-209.

There are excellent and exhaustive recent reviews of the human toxicity of PBDEs (see for example EFSA 2011, (Costa & Giordano 2011). Rather than to duplicate these efforts, the goal of this review is to

- assess whether there is evidence that BDE-209 might produce effects at doses lower than those identified by EFSA (2011) as critical for the estimation of a benchmark dose,
- investigate whether new evidence has emerged negating the critical toxicity described for BDE-209, and
- to evaluate whether there is new relevant information about BDE congeners other than the four congeners considered by EFSA (2011) as being sufficiently toxicologically tested so as to allow quantitative human risk assessment (i.e. the four congeners BDE-47, -99, -100 and -153).

In view of their relevance for conducting a mixture risk assessment (MRA), we also summarise the approaches taken to derive reference doses and health-based guidance values for specific PBDE congeners.

5.1.3.2 Study selection

In line with the goals of this sections, we searched for studies that described effects in animals below the dose of 1.7 mg/kg d, the lower 95% confidence limit of the benchmark dose (BMDL) derived for BDE-209 (EFSA 2011). These studies had to be conducted in animals.

Effects of BDE-209 on neurodevelopment which affect behaviour, have been identified as the critical endpoint relevant for human risk assessment (EFSA 2011). We attempted to locate studies negating these effects.

We also searched for studies that analysed neurodevelopmental effects for BDE congeners other than BDE-47, -99, -100 and -153.

Finally, we located overview articles and reviews on the topic of deriving reference doses and health-based guidance values for PBDEs.

5.1.3.3 Results

BDE-209, and other PBDEs exhibit a wide variety of toxic effects in mammals. BDE-209 is capable of disrupting thyroid function and of inducing neurodevelopmental effects. BDE-209 can lead to increases in liver adenoma in rats and liver adenoma and carcinoma in mice, but these effects, being related to a secondary mode of action, have been classed by the CONTAM Panel of EFSA as not relevant for human risk assessment (EFSA 2011).

The EFSA CONTAM Panel identified effects on neurodevelopment in mice which affect their behaviour as the critical endpoint. Accordingly, the Panel derived benchmark doses for BDE-209, -47, -99 and -153 based on this endpoint. In the view of the CONTAM Panel, relevant toxicity data for other BDE congeners were not available. The following sections will deal with the neurodevelopmental toxicity of PBDEs.

5.1.3.3.1 The developmental neurotoxicity of BDE-209

The developmental neurotoxicity of BDE-209 has been reviewed by Costa and Giordano (2011) who listed 14 studies with laboratory animals investigating this type of toxicity. A key study was conducted by Viberg et al. (2003) where mice were treated with BDE-209 on post-natal days (PND) 3, 10 and 19. The PND 10 exposure is normally used to test for neurodevelopmental effects of other BDEs, but BDE-209 did not produce effects at this time point. However, neurobehavioural effects with BDE-209 were seen when the mice were dosed on PND

3. Normally, "habituation" is observed in mice exposed to a new environment, defined as a decrease in locomotion, rearing and total activity, as the animals become used to the new situation. In BDE-209 exposed mice, first a decrease, and then an increase in activity was observed, which Viberg et al. (2003) interpreted as suggestive of decreased habituation. This effect was not found when dosing took place on PND 10 or 19.

The Viberg study has been criticized and this criticism was summarized by Costa and Giordano (2011). A point often raised concerns the lack of compliance with OECD guideline studies (Makris et al. 2009) and the omission of adjusting for litter effects. Further studies published by the Viberg team have extended their earlier observations (see Costa and Giordano 2011).

We located 96 articles describing the toxicity of BDE-209 and some other congeners. One of the most recent developmental neurotoxicity study with BDE-209 we identified has been conducted by (Fujimoto et al. 2011). BDE-209 was given to Sprague-Dawley rats via a diet containing the substance at concentrations of 0, 10, 100 and 1000 ppm. Reductions in the corpus callosum area in male rats were observed in the dose groups that received 100 and 1000 ppm in the diet. This study suggests that BDE-209 caused irreversible white matter hypoplasia targeting oligodendrocytes from 100 ppm onwards. This effect was accompanied by developmental hypothyroidism. This study has received comments by Biesemeier et al. (2011a) who criticised a lack of adjusting for litter effects, a failure to discuss variability of effects between studies and an omission to correlate the observed structural changes with functional changes indicative of developmental neurotoxicity. This criticism has been rebutted by Shibutani et al. (2011) with the argument that consideration of litter effects in the statistical analysis of their results did confirm their statistical significance. They also refuted the alleged variability of effects and with regard to a lack of functional assessments pointed to the usefulness of structural indicators.

One study was identified which failed to observe any developmental neurotoxicity, in contrast to the 14 studies that described such effects for BDE-209. Biesemeier et al. (2011b) conducted experiments with BDE-209 in Sprague-Dawley rats, in accordance with OECD guideline 426, with litter as the unit of statistical evaluation. In this study, BDE-209 was administered orally by gavage to dams from gestation day 6 to weaning at doses of 0, 1, 10, 100, or 1,000mg/kg/day. Treatment related effects of BDE-209 on survival and developmental neurotoxicity were not observed at doses of up to 1000 mg/kg d. The Biesemeier et al. study has been critically evaluated by Fujimoto et al. (2011) who noted the omission of measurement of thyroid-related effects, as well as histopathological parameters on neuronal migration and oligodendroglial development.

The EFSA CONTAM Panel (EFSA 2011) used the Viberg study (Viberg et al. 2007) to derive a benchmark dose (lower 95% confidence limit) of 1.7 mg/kg d.

5.1.3.3.2 Studies describing in vivo effects of BDE-209 below the benchmark dose for neurodevelopmental toxicity

The systematic review located one study which described toxic effects of BDE-209 around and below 1.7 mg/kg d. Fujimoto et al. (2011) dosed pregnant Sprague-Dawley rats with a diet containing BDE-209 at 0, 10, 100, or 1000 ppm from gestation day (GD) 10 until day 20 after delivery (PND 20). Diffuse liver cell hypertrophy and other liver toxic effects were noted at 10 ppm in the diet, equivalent to a dose of 0.7-2.4 mg/kg d.

5.1.3.3.3 Developmental neurotoxicity of BDE-183, -203 and -206

Viberg and colleagues (Viberg et al. 2006, 2009) also investigated the developmental neurotoxicity of BDE-183, -203 and -209 in mice.

BDE-183 induced behavioural effects on locomotor activity and habituation when given on PND 3, but failed to show any effects when dosing took place on PND 10 (Viberg et al. 2006).

In a series of further experiments (Viberg et al. 2006, 2009) mice were exposed to BDE-203 and -206 (>98% purity) by gavage on PND 10. Two months after dosing, they showed the same effects as described for BDE-209. Viberg et al. interpreted their observations as supporting the idea that the neurodevelopmental effects of

BDE-209 might in fact derive from its debromination products, BDE-183, -203 and -206. They based this idea on the observation that these congeners produced the same effects as BDE-209 after dosing on PND 3.

BDE-203 was the most potent of these congeners.

BDE-183, -203 and -206 are among the congeners identified as debromination products of BDE-209 in studies with mammals (see Section 5.1.1).

5.1.3.3.4 Mechanisms underlying the developmental neurotoxicity of PBDEs

The developmental neurotoxicity of BDE-47, -99 and -153 is also well described (see the reviews by EFSA 2011 and Costa and Giordano 2011). The CONTAM Panel of EFSA used the studies by Eriksson et al. (2001), Viberg et al. (2003, 2004) to derive benchmark doses for BDE-47, -99 and -153, respectively. With a lower 95% confidence limit of the benchmark dose ($BMDL_{10}$) of 12 µg/kg d, BDE-99 is the most potent of these congeners, followed by BDE-153 (83 µg/kg d) and BDE-47 (309 µg/kg d).

The mechanisms underlying these effects remain to be elucidated fully, but two, not necessarily mutually exclusive, mechanisms are discussed in the literature:

One mechanism appears to be mediated by effects on thyroid hormones. The CONTAM Panel of EFSA (2011) considered disruptions of thyroid receptor (TRß)-dependent gene expression, indicative of interference with thyroid hormone-induced neurodevelopment as the relevant mode of action.

PBDEs can also exert direct toxic effects on neuronal cells, and this is discussed as the second possible mechanism explaining developmental neurotoxicity (see Costa and Giordano 2011 and literature cited therein for a detailed discussion). Disruptions of signal transduction pathways involving protein kinase C, as well as calcium signalling are likely to be involved.

5.1.3.3.5 Deriving reference doses and health-based guidance values for PBDEs

A summary of attempts to derive reference doses and health-based guidance values for BDE-209 can be found in Costa and Giordano (2011). Various reference doses have been proposed, based on a variety of endpoints, including liver toxicity and carcinogenicity. More recent approaches focused on the developmental neurotoxicity of BDE-209 and other PBDEs, and this is now considered to be the critical toxicity.

The EFSA CONTAM Panel (EFSA 2011) has used this endpoint to derive lower 95% confidence limits for benchmark doses based on a 10% effect, $BMDL_{10}$. The classical approach to defining a reference dose or health-based guidance value would be to combine $BMDL_{10}$ with an uncertainty factor of 100 or 1000, with the aim of comparing estimated human intakes with these values. However, EFSA (2011) dismissed comparisons based on these dose metrics, for several reasons: First, it was recognized that the differences in the toxicokinetics of BDE congeners between rodents and humans were too significant so as to undermine this classical approach. Secondly, the various developmental neurotoxicity studies in rodents employed a variety of dosing regimens, including administration of single and repeated doses and this complicates straightforward comparisons on the basis of the dose metric intake or exposure on a dose per body weight basis.

For highly bioaccumulative and persistent chemicals such as PBDEs it is possible to compare body burdens, and this opens the possibility of utilizing studies with varying dosing regimens for deriving reference doses. Critical is the body burden of the animal that is associated with observed effects.

Accordingly, the $BMDL_{10}$ doses were used to estimate the body burdens of BDE-47, -99, and -153 in rodents that led to developmental neurotoxicity. By using a simple one-compartment toxicokinetic model, the EFSA CONTAM panel then determined the human intakes required to reach the same body burdens associated in rodents with neurodevelopmental effects. In this way, “critical” human exposures for BDE-47, -99 and -153 were defined as 172 ng/kg d, 4.2 ng/kg d and 9.6 ng/kg d, respectively.

In principle, this approach could be used to derive health-based guidance values for PBDEs. However, in view of some uncertainties in the toxicological data, the CONTAM panel instead opted for determining margins of exposure between these critical human intakes and those estimated to occur.

Only for BDE-209 did the EFSA Panel apply the conventional approach of comparing intake dose metric directly. A $BMDL_{10}$ of 1.7 mg/kg d was applied and compared with estimated human intakes for BDE-209.

5.1.3.4 Conclusions

BDE-209 and other congeners are developmental neurotoxicants. The CONTAM Panel of EFSA (2011) has identified developmental neurotoxicity as the critical endpoint for BDE-209, -47, -99 and -153. The Panel applied the benchmark dose approach and derived for BDE-209 a $BMDL_{10}$ for developmental neurotoxicity of 1.7 mg/kg d.

New evidence from a feeding study in rats shows that BDE-209 is capable of producing liver toxicity at doses slightly below the $BMDL_{10}$ of 1.7 mg/kg d. Diffuse liver cell hypertrophy and other liver toxic effects were noted at doses of 0.7-2.4 mg/kg d.

One study in rats failed to reproduce the neurodevelopmental effects of BDE-209 observed in numerous studies with mice, but this study has been criticised for its omission of evaluating important thyroid-related effects and structural changes in the brain.

The BDE-209 biotransformation products BDE-183, -203 and -206 also induce developmental neurotoxicity similar to that described for BDE-209 and other congeners, including BDE-47, -99, -100 and -153. This developmental neurotoxicity manifested itself as altered locomotor activity and changes in habituation. All these congeners appear to exert their developmental neurotoxicity through mechanisms involving disruption of thyroid hormone signalling and/or direct toxicity to neuronal cells involving disruption of PKC signalling and calcium homeostasis.

5.1.4 Factors affecting mixture risk assessment in human

5.1.4.1 Purpose of review

The preceding sections have identified the PBDE congeners that constitute human relevant combined exposure scenarios and have summarized the critical toxicity of PBDEs relevant to human subjects. In this section we will consider aspects relevant to a mixture risk assessment (MRA) for PBDEs.

The first question to consider is whether there is empirical evidence that various PBDE congeners can work together to produce mixture effects, ideally related to the endpoints identified as critical, i.e. developmental neurotoxicity.

If such evidence is not available, it is necessary to evaluate whether the toxicity profile established for individual PBDE congeners gives reason to believe that they might act together to elicit joint effects.

It is then required to assess whether the a consideration of BDE-209 toxicity in isolation, without taking into account co-exposures to other PBDE, might lead to underestimations of its toxicity.

Finally, we searched for approaches that have been used in the past to assess combination effects of PBDEs.

5.1.4.2 Study selection

We searched for studies of combined effects of PBDE with BDE-209 as one mixture component, as well as studies with other PBDEs that did not include BDE-209. Mixture experiments involving PBDEs with other contaminants were not considered.

Studies listed in the previous section that dealt with modes of action of PBDEs were used as the basis for evaluating the likelihood of joint effects arising from several PBDEs.

Studies that described approaches for MRA of PBDEs also qualified for inclusion in this section.

5.1.4.3 Results

5.1.4.3.1 Empirical evidence for combination effects of BDE-209 with other PBDEs

The systematic review did not locate a mixture study with BDE-209 and other PBDE congeners. The only experimental study describing combination effects between PBDEs is the paper by Tagliaferri et al. (2010) for mixtures of BDE-47 and -99.

In this study, the cytotoxicity of BDE-47 and -99 on neuronal cells was evaluated. Concentration-response relationships for the individual congeners were established, and this information was then utilised to construct additivity response surfaces for combinations of BDE-47 and -99. The additive responses were calculated by using both concentration addition and independent action (for explanations see section 6). Stronger than additive effects were observed for the combination in the range of concentrations of BDE-47 below its threshold dose, and for a wide range of BDE-99 concentrations below its half-maximal cytotoxic effect. However, at concentrations of BDE-47 near its half-maximal value and in a wide range of BDE-99 concentrations, weaker than additive effects were observed. The authors regarded the synergistic neurotoxic effects at low doses of BDE-47 as of great toxicological interest.

5.1.4.3.2 The toxicity profile of PBDE and the likelihood of combination effects

As detailed in section 5.1.3, BDE-47, -99, -153, -183, -203, -206 and -209 have been show to exhibit developmental neurotoxicity in rodents, when administered singly. There are similarities in the mechanisms underlying this toxicity; they involve disruption of thyroid hormone action and direct toxicity to neuronal cells (Costa and Giordano 2011). It is therefore plausible to assume that PBDE congeners will produce combined effects when administered jointly at sufficiently high doses.

In their evaluation of PBDEs, the CONTAM Panel of EFSA (2011) considered the potential for “additivity of different congeners” and recognized that there are “some similarities” in the effects of the various PBDE congeners, for example involving interaction with the thyroid hormone receptor.

However, the Panel came to the opinion that in view of what they referred to as “divergent responses” of different toxicity endpoints and the limited information available, the establishment of common assessment groups of PBDE with the aim of MRA is precluded. Consequently, the Panel conducted risk assessments for individual congeners, without taking account of the possibility of combined effects.

The European Union Scientific Committee SCHER (2011) was asked to evaluate whether it is appropriate to sum various PBDE congeners for the purpose of deriving environmental quality standards (EQS). SCHER considered that as long as there is no certainty about the similarity of modes of action of PBDE congeners there is no good scientific basis for using any sum parameter. However, in light of the general discussion on the risk assessment for mixtures SCHER proposed that it should be assumed that all PBDEs exhibit the same mode of action and toxicity. This would support an approach where the total concentration of PBDEs be calculated by summing up individual concentrations, with the aim of comparing the sum to the EQS. SCHER favoured this approach in view of the likely conservative estimation of the combined risks.

This approach is also in line with recent guidance of EFSA (2013) on creating common assessment groups for pesticides for the purpose of setting maximum residue limits in food. Chemicals with common adverse outcomes and common target organs should be grouped together, even when the modes of action are dissimilar.

Both the SCHER opinion (2011) and EFSA guidance (2013) support the idea of grouping PBDEs together and subjecting to mixture risk assessment (MRA).

5.1.4.3.3 Recent approaches to assessing the joint effects of PBDE

To illustrate the tiered frame work for conducting mixture risk assessment for chemicals of WHO / IPCS (see section 6.1 for a detailed description) Meek et al. (2011) chose PBDEs as a case study. The assessment was not based on congener-specific data, but instead on congener groups (tri-BDEs, tetra-BDEs etc). Critical effect doses for neurodevelopmental toxicity were used, together with upper-bounding deterministic estimates of exposure for the intake of total PBDEs for breastfed infants. Margins of exposure were then determined as approximately 300. An approach based on body burden considerations was also evaluated, but considered to be less reliable. An in-depth evaluation of PBDEs from a human health perspective was considered a low priority at the time of this analysis.

5.1.4.3.4 Conclusions

In view of the ability of PBDEs to induce common adverse outcomes, MRA is warranted. With taking into consideration that there is co-exposure of BDE-209 with other, often more toxic congeners which are formed during the biotransformation of BDE-209, the potential for underestimating the toxicity of BDE-209 is considerable.

5.1.5 References

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5.2 Environmental perspective

5.2.1 Biotransformation of BDE-209 in biota

5.2.1.1 Literature selection

The literature describing the biotransformation of BDE-209 in relevant environmental matrices and biota has been extensively reviewed in a number of recent reports (ACHS 2010, ECHA 2012, EFSA 2011, Environment Canada 2010, UNEP 2010). This material will be summarised and updated in the following section by considering the peer-reviewed literature published since those reports were drafted.

The systematic literature review database contains 56 articles classed as both relevant for ecotoxicology and related to the scientific investigation of biotransformation. Studies were considered if they were explicitly designed to investigate biotransformation experimentally, rather than just make inference about potential degradation products from biomonitoring data. The organisms that have been considered in the scientific literature are microbes (degradation in sewage sludge or sediments), including fungi (in relation to the soil-plant system), fish, birds and marine mammals. Some studies in cows and invertebrates also offered some suggestions of biotransformation in those organisms although this had not been studied directly. These will only be briefly mentioned here. The abstracts of the 56 articles retrieved by the database were reviewed for relevance and 25 articles were excluded. Six articles had not been previously cited in relevant reports. Studies carried out in rodents are relevant to wild mammals as well as human toxicology but as this evidence is already summarised within the human toxicology section (Section 5.1.1), this is not discussed further here.

With regards to the quality of the selected studies, particular attention was given to the following limitations;

- The purity of the test compound. The presence of (often unquantified) lower brominated PBDEs may act as a confounder. Preferential uptake of lower brominated congeners (as impurities in the test compound or in the exposure media) even when present below detection limits may lead to bioaccumulation to detectable levels in the exposed organisms.
- During sampling, care should have been taken to avoid photodegradation or thermal degradation.
- Analysis of PBDEs can be affected by the low solubility of some congeners. Other analytical issues have been reviewed in detail by Covaci et al. (2007) and Björklund et al.(2003) (from Environment Canada 2010). Overall, the analysis of BDE-209 in environmental samples was shown to have improved over the past decade, as demonstrated by an inter-laboratory study (Leonards & Duffek 2008).

As a result, this section will mainly focus on identifying the possible biotransformation products of BDE-209 rather than attempt to quantify the proportion of these products present in various environmental compartments.

5.2.1.2 Microbial degradation

While some studies have investigated cultures isolated from environmental media, others have been carried out with sludge, sediment or soil samples. In such cases, the use of sterilised controls is crucial to distinguish between biotic and abiotic degradation. However, significant abiotic degradation may still occur in soil samples containing high concentration of zero-valent iron, iron sulphides or manganese oxides. Finally, soil matrix and composition can also significantly affect the bioavailability of BDE-209 (e.g. adsorption to organic matter) (ACHS 2010).

5.2.1.2.1 Aerobic degradation

While microbial degradation of PBDEs has sometimes been presumed to be low and to occur only under anaerobic conditions, it has also been suggested that combinations of anaerobic and aerobic processes may be

able to fully degrade PBDEs. This paragraph will briefly summarise the sparse evidence for aerobic degradation of BDE-209.

An early screening test carried out by the Japanese Ministry of International Trade and Industry equivalent to OECD 301C using sludge inoculum detected no degradation of BDE-209 in water under aerobic conditions over a two week period as measured by biological oxygen demand (CITI 1992 as described in ECHA 2012).

ACHS (2010) succinctly recount experiments with 16 bacterial isolates from a sewage biosolid reactor carried out as part of a PhD thesis by Welsh (2008). Bacterial species fell into 3 main genera; *Aeronomas spp*, *Xanthonomas spp* and *Pseudomonas spp*. Formation of silver bromine was used to confirm the presence of free bromine but debromination products were not identified. Six isolates reduced BDE-209 concentrations at both treatment levels (40 and 80 ppb), another six at one treatment level but not both, while four isolates were not able to degrade BDE-209 under the test conditions. This study shows not only that aerobic degradation of BDE-209 can take place, but also that it occurs very quickly (under 20 minutes). Deng et al. (2011) found that *Lysinibacillus fusiformis* strain DB-1 was able to rapidly debrominate BDE-209 but degradation products were not identified.

Stiborova et al. (2008), as described in ECHA (2012), investigated the degradation of PBDEs including BDE-209 in sewage sludge under both aerobic and anaerobic conditions and found that the amount of BDE-209 had decreased by 20% after three months in the aerobic sludge slurries. Nyholm et al. (2010) investigated the biodegradation kinetics of BDE-209 in soil in laboratory microcosms over five months and reported a decrease (about 20%) that was not statistically significant. Wang et al. (2008) also investigated the debromination of BDE-209 under aerobic condition by combined *Bacillus cereus* XPB and XPC. The article is in Chinese and only little experimental detail is available from the abstract but they report that *Bacillus cereus* efficiently debrominated and degraded BDE-209 to hydroxybenzenes.

In summary, there is evidence for degradation of BDE-209 under aerobic conditions but little information on the degradation products.

5.2.1.2.2 Anaerobic degradation

5.2.1.2.2.1 Bacterial cultures

He et al. (2006) investigated the biodegradation of c-decaBDE (> 98% purity) by cultures of anaerobic bacteria, including *Dehalococcoides ethenogenes 195*, *Sulfurospirillum multivorans* and *Dehalococcoides spp*. After two months of incubation, BDE-209 was observed to degrade to non-detectable levels, while octa- and heptaBDEs became detectable in the experiment with *S. multivorans*. No degradation of BDE-209 was seen in the experiments using any of the other cultures. These findings suggest that under appropriate conditions, some strains of bacteria are capable of debrominating BDE-209.

5.2.1.2.2.2 Sludge

Although several studies inferred the potential for BDE-209 debromination during sewage treatment from grab samples at different stages of the process, the only studies that examined experimentally the biodegradation of BDE-209 in sewage sludge are those of Gerecke et al. (2005; 2006). A mass balance indicated that 3 nmol BDE-209 disappeared while 0.5 nmol of transformation products were identified. Specifically, two nonaBDEs (BDE-207, -208) and six octaBDEs were identified. Gerecke et al. suggest that the 2.5 nmol missing from their mass balance may be the result of the formation of unidentified transformation products, non-extractable BDE-209 residues or imprecisions related to the analytical method. These studies show that anaerobic bacteria can initiate debromination of BDE-209, although the residence time of BDE-209 will greatly impact its ability to anaerobically degrade.

5.2.1.2.2.3 Sediments

The majority of experimental studies investigating the anaerobic degradation of BDE-209 has been carried out with sediments. These are summarised in Table 7.

A number of studies suffered from experimental flaws. Of particular interest are the studies of Tokarz et al. (2008) and Qiu et al. (2011).

Cobalamins, such as coenzyme vitamin B12, have the ability to mediate reductive dehalogenation of halorganics and cobalamins from decaying cells have been isolated from environmental samples. Tokarz et al. (2008) used a biomimetic system with vitamin B12 to demonstrate reductive debromination at decreasing rates with decreasing bromination. They synthesized the data from their biomimetic system and sediment microcosm experiments to propose major debromination pathways as follows: BDE-209 > nonaBDEs (BDE-206, -207 -208) > octaBDEs (BDE-196, -197) > heptaBDEs (BDE-191, -184, 2 unknown heptaBDEs) > hexaBDEs (BDE-138, -128, -154, -153) > pentaBDEs (BDE-119, -99) > tetraBDEs (BDE-66, -47, -49) > triBDEs (BDE-28, -17).

In their laboratory microcosm, Qiu et al. (2011) observed the formation of nona- to hexabrominated PBDEs. Biodegradation rate was found to be correlated with the abundance of *Pseudomonas spp* and related species.

5.2.1.2.2.3.1 Mesocosm studies

The grey literature also reported on two mesocosm studies, large scale studies in semi-natural conditions in lakes.

Four experiments were carried out in a freshwater lake to examine the debromination of BDE-209 under natural field conditions as part of a Canadian government-funded project between 2007 and 2010. The peer-reviewed literature was searched and it appears that a manuscript with all experimental details has yet to be published. ECHA (2012) describe some of the preliminary findings. Two experiments involved invertebrates and fish and this is summarised in section 5.2.1.3.2. Another two studies were directly related to degradation in sediments and some experimental details are given in Table 8. Some BDE-209 breakdown products were observed in surface sediments as early as one month after deca-BDE addition. Tri-, tetra- and penta-BDEs were also observed in some treatments, but near or at detection limits in the controls. The congener pattern was similar in both experiments. BDE-205 and BDE-194 were not detected, while the predominance of BDE-206 and -207 and BDE-196, -197, -200 and -201 suggests progressive loss of bromine from the ortho- and para-positions.

Another government-funded mesocosm study using artificial indoor ponds with lake sediment is reported in ECHA (2012) although the study details are not publicly available (Feibicke et al. 2009 in ECHA 2012). Some relevant information is summarised in Table 8. The BDE-209 levels and concentrations of degradation products at the end of the study were not available but it would seem that BDE-209 was persistent in this test system.

In summary, current evidence demonstrates that BDE-209 can be debrominated both aerobically and anaerobically by some strains of bacteria to at least triBDEs.

Table 7. Experimental studies of the biodegradation of BDE-209 in sediments					
Experimental set-up	**Species**	**Test compound**	**Degradation products**	**Comments**	**reference**
Co-solvent enhanced biomimetic system, pH and reducing conditions similar to methanogenic sediments	None – vitamin B12	c-decaBDE incl. 2.0% BDE-206 1.9% BDE-207 0.9% BDE-208 on a mole fraction basis	NonaBDEs (-206, -207,-208) OctaBDEs (-196,-197) HeptaBDEs (-184, -191, 2 unknowns) HexaBDEs (-128, -138, -153, -154) PentaBDEs (-99, -119) TetraBDEs (-47, -49, -66) TriBDEs (-17, -28)		Tokarz et al. 2008
Spiked sediment microcosms with no detectable PBDEs in the dark at 22°C for 3.5 years			NonaBDE (-208) OctaBDEs (-196, -197, 3 unknowns) HeptaBDEs (-184, -191, 2 unknowns) HexaBDEs (-128, -138)	Sediment had a high organic carbon content and would have limited bioavailability to micro-organisms.	
Spiked (14 µg/g) anaerobic sediment suspension (known to contain high concentrations of BDE-209) at room temperature in the dark for 9 months			nonaBDEs	Concentrations of nonaBDEs were too low to be quantifiable. A similar decrease in BDE-209 was also found in sterilised controls. Measured BDE-209 concentration at the start of the experiment appears to be 25% of nominal concentration.	Parsons et al. 2004 and Skoczynska et al. 2005 in Environment Canada 2010
Spiked (µg/g) anaerobic sediment suspension (known to contain high concentrations of BDE-209) at room temperature in the dark			nonaBDEs were detected in the spiked samples at much higher concentrations than known background levels	No measurable decrease in BDE-209	Parson et al. 2007 in Environment Canada 2010
Spiked river sediment (10 µM) in darkness at 30°C for 90 days	Biodegradation rate was found to be correlated with the abundance of *Pseudomonas spp* and related species. No *Dehalococcoides* species were detected.	c-decaBDE (purity > 98%)	NonaBDEs, octaBDEs, hexaBDEs		Qiu et al. 2011

Table 7 cont.

Experimental set-up	Species	Test compound	Degradation products	Comments	reference
Simulation test with spiked river sediment (5 and 500mg/kg) in the dark at 22°C over 32 weeks		Mixture of unlabelled c-decaBDE (composite sample from 3 manufacturers), 97.4% purity 2.5% nonaBDE 0.04% octaBDE and ^{14}C-labelled BDE-209 (radiochemical purity 96.8%)	TetraBDEs, pentaBDEs, hexaBDEs, heptaBDEs, nonaBDEs	No statistically significant difference between levels of BDE-209 at the beginning and end of the experiment). OctaBDEs were not included in the analysis	Schaefer and Flaggs 2001a, 2001b as detailed in ECHA 2012
Microcosm using spiked sediments (100 ng, 1666.67 ng) for 189 days (temperature not reported)	DNA fingerprinting did not reveal the presence of 3 known PBDE degraders (*Dehalococcoides ethanogenes*, *Desulfitobacterium dehalogenans* and *Sulfospirillum multivorans*)	BDE-209 standard (purity not stated)	NonaBDEs	The wide variation in BDE-209 concentrations over the course of the experiment and the inconclusive data on nonaBDE make it impossible to determine whether any degradation occurred	Rheinstein 2006 as described in ECHA 2012

Table 8. Mesocosms studies of the biodegradation of BDE-209 in sediments

Experimental set-up	Species	Test compound	Degradation products	Comments	reference
Mesocosm study in an experimental lake, natural field conditions for up to `12 months Spiked (2 µg) sediment cores		^{13}C-labelled BDE-209, isotopic purity >99%	NonaBDEs, octaBDEs, BDE-119, BDE-99, -100, -33 and -32 were elevated above background level, some di-BDE congeners in the oxic /light treatment	Relative abundance of nonaBDEs, octaBDEs and hexaBDEs increased by the same proportion of BDE-209 loss	Muir and Orihel as detailed in ECHA 2012and ACHS 2010
Mesocosm study using artificial indoor ponds with lake sediment dosed with 100 ng/L, illuminated, for 191 days. Temperature varied between 10 to 25°C	Macrophytes (*Potamogeton nodosus*, *Myriophyllum spicatum*), snails (*Lymnea stagnalis*), water skaters, phyto- and zooplankton		Preliminary results (191 days) indicate that no debromination products were formed.	In the absence of a clear decreasing trend, it was concluded that BDE-209 showed no relevant degradation in sediment. It is not yet known whether the mass balance was close to 100%.	Feibicke et al. 2009 as described in ECHA 2012

5.2.1.2.2.4 Soils

Two studies examined the effects of BDE-209 on soil microbes. Other studies have considered the soil-plant system with particular reference to fungi and those are discussed in the following section 5.2.1.2.2.5.

Liu et al. (2011) examined the effects of adding different doses of BDE-209 on the soil microbial communities incubated for up to 180 days. No degradation of BDE-209 was observed. Nyholm et al. (2010) investigated the degradation of BDE-209 in laboratory soil microcosms but did not analyse for degradation products. They found that BDE-209 levels decreased by almost 20% over 160 days however this was not statistically significant. Some experimental details for these two studies are given in Table 9.

5.2.1.2.2.5 Plant-soil system

Several studies investigating the influence of plants on the degradation of BDE-209 in soils were carried out by one research group. Some experimental details are summarised in Table 9.

The addition of plants appears to have a significant effect on the biotransformation of BDE-209. Huang et al. (2010) detected several lower PBDE congeners in plant tissues. Proportions of pentaBDE and lower congeners were higher in the plant than in soil suggesting that further debromination occurred in plant tissue and/or that lower molecular weight substances are taken up more effectively. As the plants were exposed to sunlight, it is also possible that photodegradation took place within the plant. Whether microbes or plants were of most importance in the formation of degradation substances cannot be determined. Three hydroxylated PBDEs containing five bromine atoms or fewer were also detected in plant tissues at levels up to 100 µg/kg, but were not detected in soils. The same research group developed an analytical method to quantify seven hydroxylated PBDEs and successfully applied it to alfalfa exposed to BDE-209. They detected five OH-PBDEs in plant tissues, and more congeners were found in roots than in shoots (Wang et al. 2011a).

The symbiotic relationship between fungi and plant roots, known as arbuscular mycorrhizas is ubiquitous and the mycorrhiza-associated microflora is hypothesized to be an important route of metabolism of organic pollutants in soils. The role of soil micro-organism communities was considered by the same research group (Wang et al. 2011b) using a greenhouse rhizobox experiment with Italian ryegrass to investigate the effect of inoculation with arbuscular mycorrhizal fungi. The concentrations of BDE-209 after 60 days were consistently lower in the rhizoboxes inoculated with the fungus. This study suggests that the degradation of BDE-209 in soil is mediated by soil micro-organisms associated with plant roots. This is consistent with the results of an experiment carried out by Zhou et al. (2007) with cultures of white rot fungi which showed rapid degradation of BDE-209. Transformation products were not identified in this study.

Table 9. Experimental studies of the biodegradation of BDE-209 in soils

Experimental set-up	Species	Test compound	Degradation products	Comments	reference
Soil					
Spiked soil samples (1, 10, 100 mg/kg dw) at 25°C in darkness for up to 180 days	*Pseudomonas*, *Bacillus*, and uncultured bacteria dominated the bacterial community	Purity not stated	No degradation of BDE-209 was observed	Actual concentrations of BDE-209 are not reported, analysis for lower molecular weight PBDE congeners was not performed	Liu et al. 2011
Soil laboratory microcosms incubated at 20°C over 120-160 days spiked at a level of 0.5% w/w dw (similar to OECD test guideline 307)		Purity not stated	Degradation products were not investigated	BDE-209 declined by almost 20% but this decline was not statistically significant. The application method of the test substance (adsorbed to sewage sludge) may have limited its bioavailability.	Nyholm et al. 2010
Soil-plant system					
Spiked soil (≈5,000 µg/kg) under greenhouse conditions for 60 days	Italian ryegrass, alfalfa, pumpkin, summer squash, maize and radish	99.5% main impurities were 3 nonaBDEs	Soil: Mole fractions at the end of the radish experiment (highest level of transformation): Tetra (1.7%), Penta (10.3%), Hexa (24.1%), Hepta (0%) Octa (17.2%), Nona (46.6%) Plant tissue: Several lower PBDE congeners detected in plant tissue 3 hydroxylated PBDEs (up to 100 µg/kg dw) OH-BDE (-7, -17, -28, -47) in alfalfa	Average BDE-209 loss 20%. The overall pattern of lower brominated congener formation is obscured by the fact that only a small number of isomers were determined in each congener group. For hydroxylated compounds, differences in the congeners detected were observed between root and shoots samples.	Huang et al. 2010 and Wang et al. 2011a
Rhizobox experiment (≈3,500 ng/g BDE-209) in greenhouse conditions for 60 days	Italian ryegrass inoculated with arbuscular mycorrhizal fungus (*Glomus mosseae*)	Purity not stated Main impurities appear to be 3 nonaBDEs	Soil: di-BDE(-7, -15), tri-BDE(-28), tetra-BDE (-47, -49, -66), Hepta-BDE (-191), Octa-BDE (-196, -197), Nona-BDE (-206, -207, -208) Plant: A total of 24 lower brominated PBDEs (di- through nona-) were detected in the plant samples	Concentrations of BDE-209 were higher in mycorrhizal roots than in non-mycorrhizal controls	Wang et al. 2011b

5.2.1.3 Transformation in aquatic species

5.2.1.3.1 Fish

Several laboratory studies have investigated the transformation products of BDE-209 in several species of fish (Table 10). Studies where fish were exposed to a mixture of PBDEs including BDE-209 are difficult to interpret and are not considered in this report. Four studies had not been previously included in reports (Chen et al. 2012; Feng et al. 2012; Luo et al. 2013; Zeng et al. 2012). In addition, two studies reported *in vitro* investigation of BDE-209 debromination (Table 11).

Overall, these studies demonstrate that fish can absorb BDE-209 from their diet and that it can be metabolised to lower brominated congeners (at least hexa- and heptaBDEs) as well as methoxylated and hydroxylated PBDEs. Further the formation of precursors such as nona- and octaBDEs could provide an ongoing source of lower brominated congeners. *In vitro* studies confirm the existence of species differences in the extent of transformation as well as the potential role of thyroxine deiodinase enzymes in catalysing debromination. The role of cytochrome P450 or glutathione-S-transferases enzymes cannot be ruled out in debromination and is suspected in the formation of hydroxylated products.

Table 10. In vivo experimental studies of the biodegradation of BDE-209 in fish					
Experimental set-up	**Species**	**Test compound**	**Degradation products**	**Comments**	**reference**
Dietary exposure (between 7.5 and 10 mg/kg bw/day) at summer outdoors temperature (Sweden) for 120 days	Rainbow trout (*Oncorhynchus mykiss*)	Commercial flame retardant (DOW FR-300-BA), actual composition not given	Concentrations of some hepta-, octa- and nona-BDE congeners were found to increase in both muscle and liver in exposed fish but not controls	Presence of lower congeners could be the result of a metabolic process or efficient absorption of trace amounts initially present.	Kierkegaard et al. 1999
Dietary exposure (1g/day/fish) at 22°C for 60 days followed by 40 days depuration	Common carp (*Cyprinus carpio*)	>98% pure No information was given on the identities of impurities	1 unknown pentaBDE hexaBDEs (-154, 155, 1 unknown) 2 unknown heptaBDEs 1 unknown octaBDE	No BDE-209 in whole fish tissues. Levels of total PBDEs were higher in livers than in whole fish	Stapleton et al. 2004
Dietary exposure (1% bw/day for 5 days/week) for 5 months	Rainbow trout (*Oncorhynchus mykiss*)	98.7% purity	nonaBDEs (-206, -207, -208) (37%) 6 octaBDEs (incl, BDE-201 and -202) (32%) heptaBDEs (mostly BDE-188 and 3 others) (2%) hexaBDEs (< 2%)	Levels of total PBDEs were higher in livers than in whole fish	Stapleton et al. 2006
Fish injected with 400 ng/g BDE-209 kept 6-7°C for 7 weeks	Atlantic tomcod (*Microgadus tomcod*)	≈96% purity Impurities included nonaBDEs, octaBDEs, heptaBDEs, hexaBDEs	BDE-47, -99, -100 (80%) nonaBDEs (-206, -207, -208) octaBDE (-203, 3 unknowns) BDE-118 BDE-138 2 MeO-tetraBDEs	Pre-treatment with PCB-126, a potent cytochrome P4501A inducer. Livers of control fish also contained 12 identifiable PBDEs. The pattern of congeners could be explained by the uptake of impurities. This fish species exhibited limited capacity to metabolise BDE-209 to lower congeners.	Lebeuf et al. 2006
Intraperitoneal injection (100 or 500 ng/g fresh weight), kept at 15°C for 28 days	Rainbow trout (*Oncorhynchus mykiss*)	Commercial BDE, purity > 98%	NonaBDEs (-206, -207) OctaBDE (-197) heptaBDEs (-183, -184) pentaBDEs (-85, -99, -100, -119, -126) tetraBDEs (-47, -49, -66, -71, -77) triBDE (-28) diBDEs (-7, -15) monoBDE (-3) MeO-tetraBDEs (-47, -49) MeO-pentaBDE (-100)	Highest concentrations in the liver, followed by blood then muscle. Exposure route is not environmentally realistic and study used internal doses around 2 orders of magnitude higher than those typically found in environmental fish samples. Fish appear to have only been fed once a week.	Feng et al. 2010a, 2010b

Table 10 cont.

Experimental set-up	Species	Test compound	Degradation products	Comments	reference
Dietary exposure (8-10 µg/g of food at 5% bw/day) for 28 days followed by 14 days depuration	Fathead minnow (*Pimephales promelas*)	98% pure	Dominant PBDEs detected were octaBDEs (-201, -202) heptaBDEs (-179, 188) hexaBDEs (-154, 1 unknown) pentaBDEs also found	Lack of information on the test substance does not allow to rule out lower congener accumulation due to trace impurities	Noyes et al. 2011
Dietary exposure (150 µg/day/fish) over 20 days at 22°C	Common carp (*Cyprinus carpio*)	c-decaBDE (purity not reported)	BDE-47, BDE-154, -155, -149, -188, -179, -201 and -202	No methoxylated or hydroxylated BDEs were found in serum samples	Zeng et al. 2012
Dietary exposure (0.1, 1, 2 µg/g diet) for 30 days	Lake whitefish (*Coregonus clupeaformis*)	Not reported	NonaBDEs (-206, -207, -208)	Detection of BDE-209 in control fish was due to its presence in the base diet. Concentrations were higher in the liver than in carcasses	Kuo et al. 2010
Flow-through exposure (1 to 1000 ng/L) for up to 60 days at 25°C	Japanese medaka (*Oryzias latipes*)	Not reported	BDE-28, -47, -99, -100, -154, -155	The purity is not reported so it is difficult to conclude whether this fish species has a different metabolic pattern or whether this is due to the exposure route or impurity in test compound.	Luo et al. 2013
Embryos exposed in water (0, 0.08, 0.38 and 1.92 mg/L) in water at 28°C until 14 days post-fertilization	Zebrafish (*Danio rerio*)	Purity > 98%	NonaBDEs (-206, -207) OctaBDE (-196, -197)	The limited metabolites of BDE-209 detected in our study compared with previous studies suggest lower metabolic capability in larvae, but it may also be due to the different exposure times and species utilized.	Chen et al. 2012
Intraperitoneal injection at 5 nominal gradient concentrations from 50 to 1000 ng/g ww, kept at 15°C for 21 days	Rainbow trout (*Oncorhynchus mykiss*)	Purity > 98%	NonaBDEs (-206, -207) OctaBDE (-197) heptaBDEs (-183, -184) pentaBDEs (-85, -99, -100, -119, -126) tetraBDEs (-47, -49, -66, -71, -77) triBDE (-28) diBDEs (-7, -15) monoBDE (-3) MeO-BDEs (- 47, -49, -68, - 100, -103) OH-BDE (-28, -42)		Feng et al. 2012

Table 11. In vitro experimental studies of the biodegradation of BDE-209 in fish

Experimental set-up	Species	Test compound	Degradation products	Comments	reference
Microsomal preparations (15 pmoles BDE-209/mg protein) for 1 and 24 hours at 25°C	Rainbow trout (*Oncorhynchus mykiss*)	98.7% purity	nonaBDEs octaBDEs		Stapleton et al. 2006
	Common carp (*Cyprinus carpio*)		nonaBDEs octaBDEs heptaBDEs hexaBDEs		
Microsomal preparations (1 nmol BDE-209 for 10 mg protein) for 24 hours at 25°C	Rainbow trout (*Oncorhynchus mykiss*)	Not reported	Major metabolites were heptaBDE (-183) hexaBDE (-154) pentaBDE (-101) tetraBDE (-49) and BDE-47 in *C. carpio*	Metabolite formation rates were generally 10 to 100 faster in *C. Carpio*. Carp liver demonstrated a preference for meta-debromination, while the other two species debrominated meta- and para-bromine atoms to an equal extent. Carp exhibited a preference for meta-deiodination of the thyroid hormone thyroxine consistent with meta-debromination.	Roberts et al. 2011
	Common carp (*Cyprinus carpio*)				
	Chinook salmon (*Oncorhynchus tschwatcha*)				

5.2.1.3.2 Invertebrates

It is commonly assumed that it is unlikely that invertebrates are able to debrominate BDE-209 given that they typically have less well developed biotransforming enzymes than vertebrates. This has not been studied extensively experimentally, and the literature search only identified three studies that provide circumstantial evidence that debromination may be possible in some invertebrate species.

La Guardia et al. (2007) examined debromination in the field. PBDE congener profiles were tracked from a wastewater treatment plant (sludge) to receiving stream sediments and associated aquatic biota. BDE-209 and 23 additional PBDEs were detected and congener profiles resembled the commercial penta- and deca-formulations, suggesting minimal debromination during wastewater treatment. Crayfish (*Cambarus puncticambarus spp*) contained tri- through deca-PBDEs, including congeners not detected in the commercial mixture, sludges or sediments (BDE-179, -184, -188, -201, and -202). A previous in study identified these as BDE-209 debromination products.

Klosterhaus and Baker (2010) conducted a bioaccumulation experiment in which the marine polychaete worm *Nereis Wrens* was exposed to a commercial mixture of deca-BDE (purity >85%) in spiked sediments, in spiked food, or in field sediments. Bioaccumulation from spiked substrate demonstrated that BDE 209 accumulates in this species. Of particular interest was the approximately 10 times higher bioavailability of BDE-208 compared with other congeners. This could indicate biotransformation rather than higher bioavailability.

Riva et al. (2007) were investigating the genotoxicity of BDE-209 using a zebra mussel (*Dreissena polymorpha*) model. Incidentally, chemical analyses of zebra mussels exposed to BDE-209 revealed several unidentified peaks. Although no quantitative determinations were carried out, the retention time of these peaks coincided with that of three hepta-, three octa-, and three nona-BDE congeners. It should be noted that only two nona-BDEs were found as impurities of BDE-209 mixture employed for this study.

As mentioned in section 5.2.1.2.2.3.1, two freshwater lake mesocosm studies were carried out as part of a Canadian government-funded project (Muir and Orihel as described in ECHA 2012). As this is a field study, observations could be related to abiotic, microbial, invertebrate and fish metabolism concurrently. Full results are not yet publicly available. Zooplankton in the mesocosm that received the highest dose of a commercial BDE-209 contained tetra- through to nonaBDEs in higher amounts than the control after the first year.

5.2.1.3.3 Marine mammals

McKinney et al. (2011) assessed the oxidative and reductive biotransformation of BDE-209 using an *in vitro* system based on liver microsomes from various arctic marine-feeding mammals; namely the polar bear (*Ursus maritimus*), beluga whale (*Delphinapterus leucas*), and ringed seal (*Pusa hispida*). They found no evidence of simply debrominated metabolites despite depletion of the parent compound and suggested that further research is needed to identify transformation products.

5.2.1.4 Transformation in terrestrial species

5.2.1.4.1 Birds

Although the congener patterns in the eggs or plasma of several bird species has been investigated, to our knowledge there is only two experimental studies that examined the biodegradation of BDE-209 *in vivo* in birds. Van den Steen et al. (2007) studied the tissue distribution and metabolism of BDE-209 in European Starlings (*Sturnus vulgaris*). Adult male birds were exposed via implanted silastic tubes. No lower PBDE congeners were detected in any samples of blood during the exposure period, however other PBDE congeners including hexa- and heptaBDEs were found in liver and muscle samples. Many of these congeners were also present in the control birds but there were marked differences between exposed and control birds for some of the octa- and nonaBDEs. In a very recent study, Letcher et al. (2014) examined the metabolism of BDE-209 in a raptorial species, American kestrels, following dietary exposure. They detected meta- and para-debromination products in the plasma, liver and fat of exposed birds, including nona-, octa- and heptaBDEs. Higher hepatic

EROD activity in the exposed birds suggested that debromination was mediated via the cytochrome P450 (CYP) 1A1.

The literature search identified an *in vitro* study that had not been previously reported. Chabot-Giguere et al. (2013) used liver microsomes of Montreal-breeding ring-billed gulls (*Larus delawarensis*). No depletion of BDE-209 was observed in this 90-min assay. In contrast to the *in vivo* study conducted by Letcher et al., the authors concluded that that CYP isoenzyme-mediated dehalogenation is not likely to be a substantial metabolic pathway and suggested further investigation of the expression and activity of deiodinases. Debromination products detected in the liver of ring-billed gulls in biomonitoring studies could have been indicative of dietary and environmental exposure rather than biotransformation.

In summary, there is now evidence that debromination of BDE-209 occurs at least in some bird species. The sparse evidence suggests that there may be differences between species with regards to BDE-209 metabolism.

5.2.1.4.2 Cows

In one study, lactating cows were fed a diet naturally contaminated with PBDEs. Kierkegaard et al. (2007) observed that pattern of congeners in the feed was the same as in the faeces, indicating that no changes took place in the rumen. However, other tissues including milk fat were enriched in nona- , octa- and heptaBDEs suggesting metabolic reductive debromination.

5.2.1.5 References

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5.2.2 Likely environmental exposure to PBDE mixtures, including after biotransformation

5.2.2.1 Literature selection

In this section, we firstly indexed all the individual PBDE congeners that were examined in all the database studies that reported an exposure scenario, this provided us with an overview identifying which congeners have been studied and in which exposure scenarios. Secondly, we then analysed a selection of studies to provide a snapshot of recent data. Exposure data from these selected studies was compiled for possible use in environmental mixture risk assessment (Section 6.3). The purpose of this section is to provide information on exposure levels to which biota are exposed considering both PBDEs present in the environment (see Section 5.2.2.2.2) and PBDEs that may be present in organisms, including after biotransformation (see Section 5.2.2.2.3).

Consideration was limited to: studies providing exposure data on environmental compartments (excluding air, dust and compartments specifically related to human exposure) and organisms other than humans; studies that included BDE-209; and studies rated as minor relevance and with full text not in English were excluded (see database content report, Section 4).

Temporal trends in PBDE concentrations are known and are to be expected, so we have prioritized recent publications with the aim of reflecting the current exposure situation. Analytical techniques and availability of high quality standards has also improved over time, so that more recent studies are also generally, but not always, of better quality. The number of congeners studied has also increased over time, and we have preferred those studies that examined the widest number of congeners, which are therefore also the more recent studies. Publication year was used as a proxy for the date of sample collection and cases when this was strikingly different are noted.

The large number of papers identified meant an analysis of the experimental quality of every paper was not attempted.

5.2.2.1.1 Technical considerations

The main task for this section of the report was the identification of congeners examined in each study. This was complicated by extreme differences in the reporting of the congeners that were examined by each study. Many papers failed to separately identify the congeners employed as standards (although those used as internal standards were usually identified), the congeners for which the method was validated (for example by reporting a limit of detection- LOD), congeners actually detected in the experimental samples, and congeners that contributed the most to the mass of PBDE congeners present. Many studies reported a sum of PBDEs, Sigma PBDE or ∑PBDE, often of 8 or 11 PBDEs and did not provide individual congener level data, even when they had measured it. There is a clear space limitation on the reporting of congener numbers in abstracts and keyword. The issue is not restricted to PBDEs as the indexing of most studies of multiple chemicals is not systematic. In some cases all congener information was relegated to the supplementary information, or was simply not provided.

One positive aspect is that the congener numbers, usually referred to as IUPAC or BZ numbers and attributed to the system provided by Ballschmiter and Zell, were universally employed and appeared to be used consistently and unambiguously (Ballschmiter & Zell 1980). It seems that CAS numbers have not been provided for each PBDE congener, only for the ten homologue groups and for the commercial products.

These technical aspects prevent the easy retrieval of data at the individual congener level, and therefore necessitate approaches such as employed in this report when relevant papers are identified by a wide-focus keyword search and then the results are manually indexed. This approach can be comprehensive, but is laborious, depends on the initial search, and is not readily updated.

5.2.2.2 Results

5.2.2.2.1 Overall survey, congeners examined in 304 studies

The systematic search identified 304 papers with data on environmental exposure to PBDE congeners, including BDE-209. Each paper was classified as to the environmental compartment and species or species group studied, and the congener number of each PBDE analyzed in the study was compiled. This data is provided in the data file: EXCELFILE ECO_1.xlsx. This allows the identification of the number of congeners examined in each study, and also the number of studies in which each congener was examined.

These results are discussed collectively in the remainder of the section before being analyzed in groups based on environmental compartments and organisms (Section 5.2.2.2.2).

The identified articles were published between 1998 and 2013, and the number of congeners examined in each study are shown graphically in Figure 2. Over time, the number of congeners examined per study has increased, and peaked in 2012 (discounting 2013, for which the publication year is ongoing at the time of this report). The study that examined the highest number of congeners was from 2012 and studied 83 congeners (Li et al. 2012), followed by a study of 53 congeners (Wei et al. 2012). The Li et al. study is described below. All of the other studies examined fewer than 50 congeners. Figure 3 shows that the average number of congeners examined in studies published in 2012 was 17 (median, 19.8 mean); this finding has consequences for the power of typical studies to cover all 209 PBDE congeners, for situations when such information might be desirable. Essentially, it should not be expected that this information is available from a large number of studies, and any conclusions about the set of 209 PBDEs, or a large number of congeners, will be inevitably have to be based on a small number of studies. This is not a criticism of the individual studies, whose purpose and aim was not normally to provide a full congener profile.

Li et al examined 83 PBDE congeners in samples from 28 landfills and dumpsites across Canada (Li et al. 2012). They reported that PBDEs were detected in almost all samples, with total concentrations of up to 1,020 ng/L (mean 166 ng/L), giving an estimated average PBDE loading from urban landfill to the environment of 3.5 tonnes/year. Although 83 congeners were targeted, only 59 congeners were monitored in all samples, and only nine congeners (BDE-47, -99, -100, -153, -154, -183, -206, -207, and -209) were frequently detected in most samples. The sum of these 9 congeners constituted more than 99% of the total PBDEs measured. BDE-209 was the dominant congener (52% of total) and there were strong correlations between certain congeners for example, the major congeners in the penta-BDE commercial mix (BDE-47, -99 and -100).

Over all 304 studies, the eight most frequently studied congeners (Figure 4) were (in numerical order of congener no.):

BDE-28, -47, -99, -100, -153, -154, -183, -209.

114 congeners were not examined in any of the studies we identified, their congener numbers were:

BDEs -4, -5, -6, -9, -14, -16, -19, -20, -21, -23, -24, -26, -27, -29, -34, -36, -38, -39, -40, -41, -43, -44, -45, -46, -48, -50, -52, -53, -55, -56, -57, -58, -59, -61, -62, -63, -64, -65, -67, -68, -69, -70, -72, -73, -74, -76, -80, -83, -84, -86, -87, -88, -89, -90, -91, -92, -93, -94, -95, -96, -98, -103, -106, -107, -108, -109, -110, -111, -112, -113, -114, -117, -121, -122, -123, -124, -125, -127, -129, -130, -131, -132, -133, -134, -135, -136, -137, -141, -142, -143, -145, -146, -147, -148, -150, -151, -152, -157, -158, -159, -161, -162, -163, -164, -165, -167, -168, -172, -174, -175, -178, -186, -187, -193

Figure 2: Scatter plot showing number of congeners examined per study over time

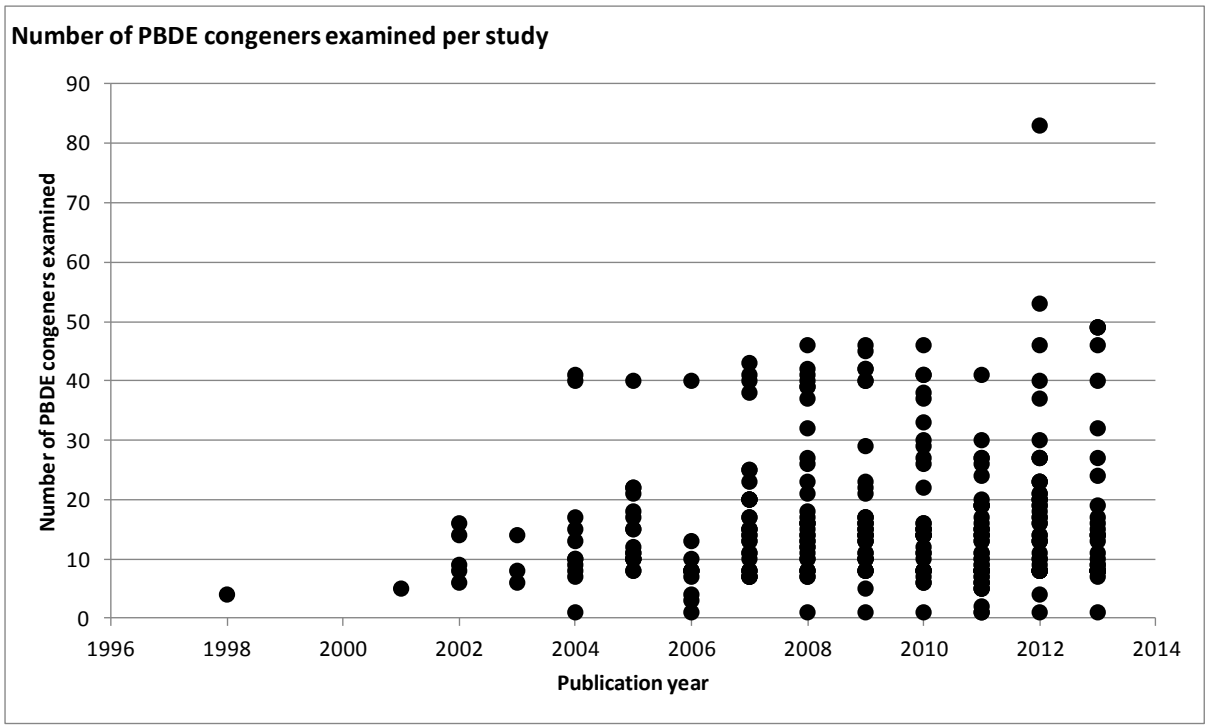
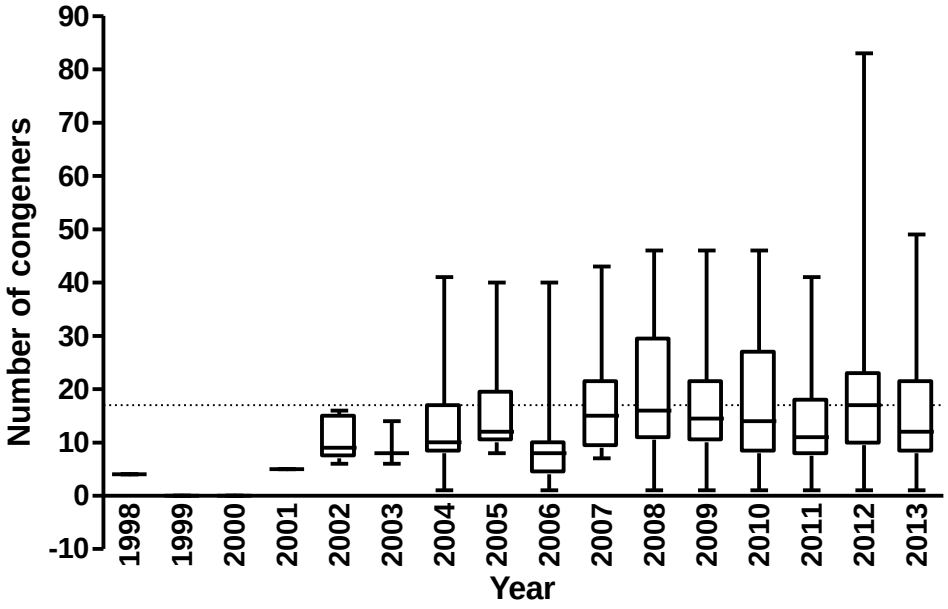


Figure 3: Box and whisker plots of number of congeners examined per study, per year

A. Box and whisker plots showing the distribution of the number of congeners studied per year.



B. Expanded view of data in A; the y-axis is truncated at 30 to allow the central portion of each 'box', representing the 25, 50 (median) and 75th percentiles, to be clearly seen.

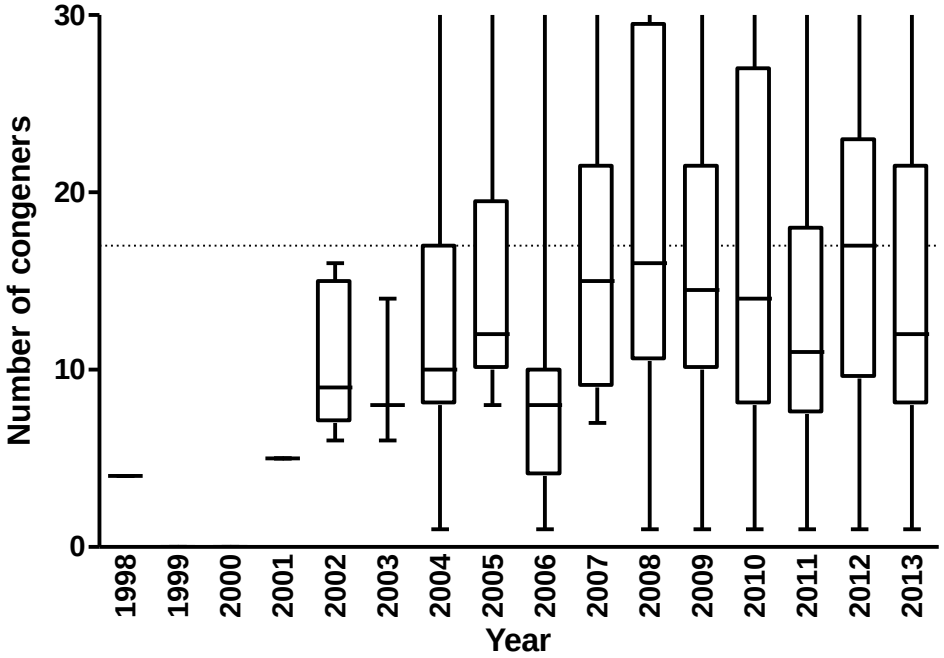
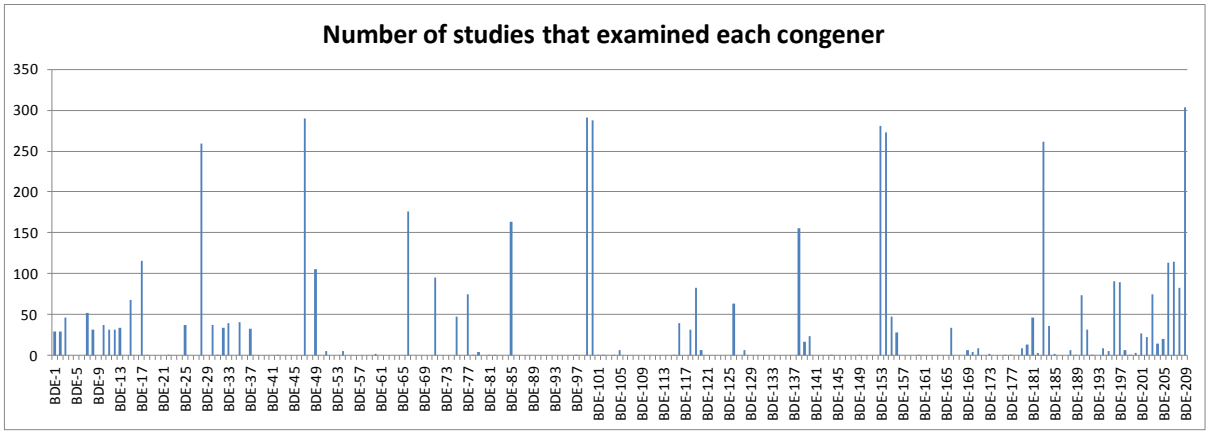
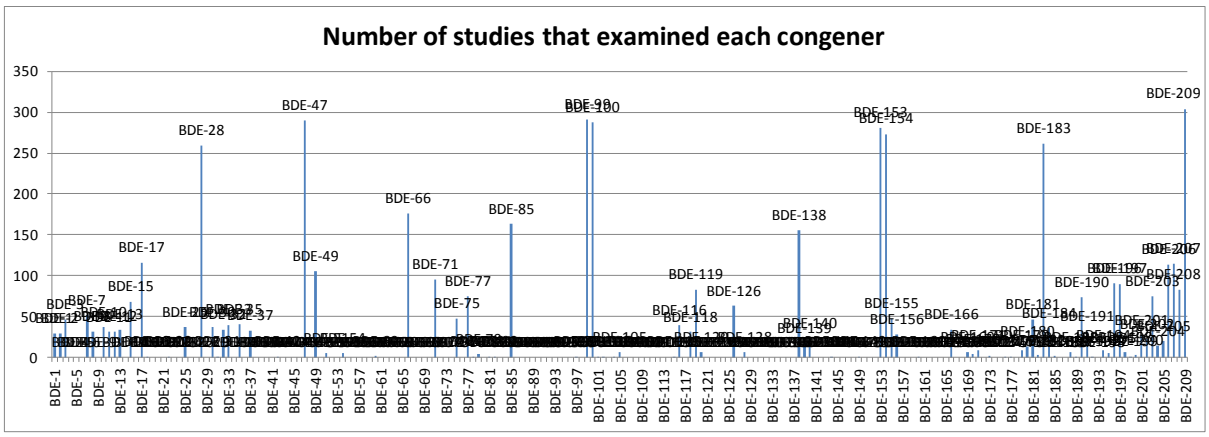


Figure 4: Number of studies that examined each congener

A. number of studies that examined each congener



B. same data as A, labelled with congener numbers



5.2.2.2.1.1 Consensus profiles

To facilitate comparison of studies, a consensus set of 8 PBDE congeners has been proposed: BDE-28, -47, -99, -100, -153, -154, -183, -209 (Law et al. 2008). These congeners are the same as the set of 8 congeners that were most frequently studied in the identified studies for this report, see above and are regarded as relevant in human toxicology. At the time, Law et al. estimated that around half of all studies had measured the consensus set, however although this set might be suitable for comparison of studies and for monitoring the use of the three commercial mixtures (tetra, octa and deca BDEs), it should not be considered sufficient for all purposes since it is not likely to be suitable for the study and monitoring of the biotransformation products of c-decaBDE, such as nona-brominated congeners, or indeed any breakdown products of lower brominated congeners. Monitoring of the commercial mixtures may become less of a priority as their use has been, or becomes restricted, through for example the Stockholm Convention, and focus should shift to monitoring the long term fate of PBDEs in the environment and in biota. The consensus set may be useful as a minimum, and for the purpose of comparing studies, but other congeners certainly merit study.

5.2.2.2.1.2 Hydroxylated and methoxylated PBDE congeners

Systematic review identified 6 studies that looked at either hydroxylated or methoxylated PBDE congeners, or both. A narrative review of the content relating to OH and MeO congeners is provided below, but exposure data was not compiled. Table 12 lists the number and identities of the congeners tested.

Verreault et al. 2005 measured 15 hydroxylated (OH-PBDEs) and 15 methoxylated (MeO-PBDEs) congeners in plasma from two top predators from the Norwegian Arctic: glaucous gulls and polar bears (Verreault et al. 2005). Around half of the target congeners were detectable and MeO congeners of BDE-47 and BDE-49 were the most dominant. OH and MeO congeners were more commonly detected in gulls than in polar bears and the sum of measured MeO PBDEs was 0.3 - 4.3 ng/g ww (glaucous gulls) and up to 0.17 ng/g ww (polar bears). MeO-PBDEs were considered to occur in wildlife as a consequence of accumulation from natural sources (e.g., via formation in sponges and green algae) whilst OH-PBDEs could be of natural origin or be metabolically derived from precursor PBDEs. Verrault et al also proposed that, although not yet shown, MeO-PBDEs might also be formed metabolically, by methylation of OH-PBDEs or methoxylation of precursor PBDEs.

Verreault et al. 2007 examined eggs and plasma from glaucous gulls, and measured 14 OH and 15 MeO PBDEs (Verreault et al. 2007). In egg yolk, 4 MeO congeners (of BDE-17, -42, -47) comprised around 10% of total PBDEs.

Fernie and Letcher 2010 examined 14 hydroxylated and 14 methoxylated PBDEs in plasma from Peregrine falcon nestling in the Canadian Great Lakes basin, and reported detection of low ppb levels of putative OH-PBDE metabolites, dominated by 6'-OH-BDE47 (Fernie & Letcher 2010). Methoxylated congeners were undetectable.

Nordlof et al. 2010 examined 3 naturally occurring methoxylated congeners (of BDE-47 and -68) in the eggs of white-tailed sea eagles breeding in different regions of Sweden (Nordlof et al. 2010). Levels of MeO congeners ranged from 6-270 ng/g lipid weight; for comparison, these levels exceeded those of BDE-209, which was undetectable in these samples, whilst the dominant congener was BDE-47 (280-2600 ng/g lipid weight).

Baron et al. 2013 studied 8 naturally occurring methoxylated congeners in marine biota (primary secondary and tertiary consumers) from Chile (Baron et al. 2013). Total MeO congener levels ranged from not detected to 118 ng/g lipid weight and were dominated by methoxylated BDE-47 and BDE-68). Values showed significant differences both between and among species, which the authors associated with the different metabolic capacity of the species to incorporate or transform different MeO-PBDE congeners and with variability in the production of methoxylated congeners from natural sources.

Mizukawa et al. 2013 examined 23 hydroxylated and 15 methoxylated PBDE congeners in blood samples from terrestrial mammals in Japan. (Mizukawa et al. 2013). Samples from all species had a high proportion of BDE-209, suggesting exposure to municipal waste and soil containing higher levels of deca-BDE products. In all species, congener profiles were dominated by 6OH-/MeO-BDE47 and 2OOH-/MeO-BDE68. Total levels of 23 OH-PBDEs were greater than total levels of 15 MeO-PBDEs, and could exceed the total of 11 PBDE congeners that

were also studied, for example in cats (pg/g whole blood wet weight): ∑PBDE(11 congeners), 580; ∑OH-PBDE(23 congeners), 720; ∑MeO-PBDE(15 congeners).

In summary, methoxylated and hydroxylated congeners have been examined in only a few studies. In species at higher trophic levels, methoxylated congeners may reflect biotransformation in lower trophic levels rather than biotransformation in the examined species. Hydroxylated congeners have generally been detected at lower levels than methoxylated congeners. Neither congener type is routinely studied and the full range of possible hydroxylated and methoxylated congeners has not been studied. The most detailed study examined 23 OH BDEs and 15 MeO PBDEs (Mizukawa et al. 2013). The congeners measured to date are dominated by methoxylated and hydroxylated congeners of BDE-47 and products of higher BDEs have not been studied. Separate toxicology data is not generally available for PBDE metabolites, and the toxic effects measured in a toxicity study are already likely to be a composite of the effects of the parent compound and of any metabolites formed during the study.

Table 12: Studies reporting hydroxylated and methoxylated BDE congeners

Reference	Hydroxylated congeners		Methoxylated congeners	
	n	Identities	n	Identities
(Verreault et al. 2005)	15	4'OH-BDE17, 6'OH-BDE17, 4OH-BDE42, 6OH-BDE47, 3OH-BDE47, 5OH-BDE47, 4'OH-BDE49, 6'OH-BDE49, 2'OH-BDE68, 6OH-BDE85, 6OH-BDE90, 6OH-BDE99, 2OH-BDE123, 6OH-BDE137	15	6'MeO-BDE17, 4'MeO-BDE17, 2'MeO-BDE28 , 4MeO-BDE42, 6MeO-BDE47, 3MeO-BDE47, 4'MeO-BDE49, 5MeO-BDE47, 6'MeO-BDE49, 2'MeO-BDE68, 6MeOBDE85, 6MeO-BDE90/6MeO-BDE99, 2MeO-BDE123, 6MeO-BDE137
(Verreault et al. 2007)	14	6'OH-BDE17, 4'OH-BDE17, 4OH-BDE42, 6OH-BDE47, 3OH-BDE47, 5OH-BDE47, 6'OH-BDE49, 4'OH-BDE49, 2'OH-BDE68, 6OH-BDE85, 6OH-BDE90, 6OH-BDE99, 2OH-BDE123, 6OH-BDE137,	15	6'MeO-BDE17, 4'MeO-BDE17, 2'MeO-BDE28, 4MeO-BDE42, 5MeO-BDE47, 6MeO-BDE47, 3MeO-BDE47, 4'MeO-BDE49, 6'MeO-BDE49, 2'MeO-BDE68, 6MeO-BDE85, 6MeO-BDE90, 6MeO-BDE99, 2MeO-BDE123, 6MeO-BDE137
(Fernie & Letcher 2010)	14	4OH-BDE17, 6'OH-BDE17, 4OH-BDE42, 3OH-BDE47, 5OH-BDE47, 6OH-BDE47, 4'OH-BDE49, 6'OH-BDE49, 2'OH-BDE68, 6OH-BDE85, 6OH-BDE90, 6OH-BDE99, 2OH-BDE123, 6OH-BDE137		4MEO-BDE17, 6'MEO-BDE17, 4MEO-BDE42, 3MEO-BDE47, 5MEO-BDE47, 6MEO-BDE47, 4'MEO-BDE49, 6'MEO-BDE49, 2'MEO-BDE68, 6MEO-BDE85, 6MEO-BDE90, 6MEO-BDE99, 2MEO-BDE123, 6MEO-BDE137
(Nordlof et al. 2010)			3	6MeO-BDE47, 2'MeO-BDE68, 5Cl-6-MeO-BDE47
(Baron et al. 2013)			8	6MeO-BDE47, 5MeO-BDE47, 4MeO-BDE49, 2MeO-BDE68, 5MeO-BDE99, 5MeO-BDE100, 4MeO-BDE101, 4MeO-BDE103
(Mizukawa et al. 2013)	23	4'OH-BDE17, 2'OH-BDE28, 3'OH-BDE28, 4'OH-BDE42, 3OH-BDE47, 5OH-BDE47, 6OH-BDE47, 4OH-BDE49, 2'OH-BDE68, 4OH-BDE90, 5'OH-BDE99, 6OH-BDE99, 5'OH-BDE100, 4'OH-BDE101, 4'OH-BDE103, 6OH-BDE140, 3OH-BDE154, 6OH-BDE157, 6OH-BDE180, 4OH-BDE182, 4OH-BDE187, 4OH-BDE188, 4'OH-BDE201	15	4'MeO-BDE17, 2'MeO-BDE28, 3'MeO-BDE28, 4'MeO-BDE42, 3MeO-BDE47, 5MeO-BDE47, 6MeO-BDE47, 4MeO-BDE49, 2MeO-BDE68, 4MeO-BDE90, 5'MeO-BDE99, 6MeO-BDE99, 5'MeO-BDE100, 4'MeO-BDE101, 4'MeO-BDE103

5.2.2.2.1.3 Temporal trends

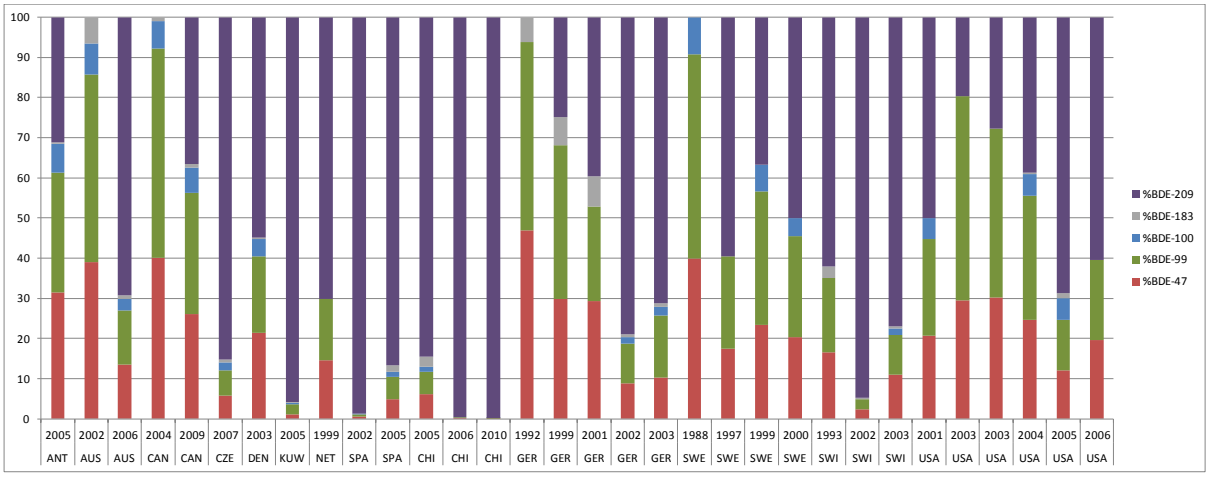
A detailed analysis of temporal trends was outside the scope of this project, and it is clear from the data picture provided by our survey of 304 papers that data for many congeners will not be available, and is unlikely to be available in studies that can be readily compared over time. Therefore only one paper is reviewed here and the remainder of our analysis is focused on recent publications that have the best chance of reflecting current exposure levels. Care must be taken however in concluding that this is the case, and the fact that the exposure situation is highly likely to change over time calls for any mixture risk assessment to be based on contemporary data and to be updated over time.

Yang et al. summarized levels of 5 PBDEs (BDE-47, -99, -100, -183, -209) in sewage sludge (an environmental compartment which may reflect contemporary PBDE usage) for multiple countries and time periods (Yang et al. 2011). This data is shown in Figure 5 with the level of each congener expressed as a percentage of the total. In general BDE-100 (blue) and BDE-183 (grey) make small contributions, however the contributions for BDE-47 (red), BDE-99(green) and BDE-209(purple) vary greatly. Within each country where a time trend may be seen (Figure 5A, China, Germany, Sweden, Switzerland, USA) the contribution of BDE-209 has increased, and that of BDE-47 and -99 has decreased. Note that the time periods shown vary between countries: China, 2005-2010; Germany, 1992-2003; Sweden, 1988-2002; Switzerland, 1993-2003; USA, 2001-2006. Figure 5B does not show a clear pattern over time, presumably because the temporal trends are different within each of the countries included.

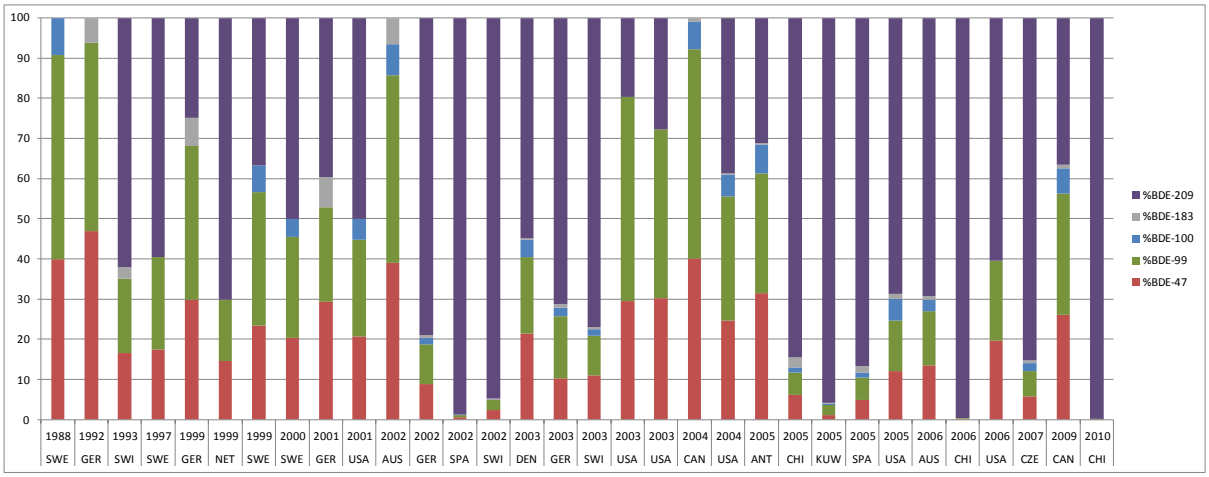
The most important observation however is that the mixture exposure scenario has changed with time, and there is significant geographical variation.

Figure 5: percentage congener profiles (weight basis)

A. Percentage congener profile in sewage sludge (Yang et al. 2011); ordered by country then year



B) Percentage congener profile; ordered by year then country



5.2.2.2.2 Selected analysis, PBDE exposure levels in environmental compartments and organisms

Details of measured PBDE exposures is now given according to a sub-sample of all the identified studies from literature groups based on environmental compartment and organism. The groupings used are listed in Table 13. For the purposes of environmental mixture risk assessment (MRA) (section 6.2) we compiled data for 3 studies if available, for each compartment/organism group. 51 studies were considered for use, and 35 were suitable. The main reason for not including a study was the failure to report data for individual congeners even when this had been measured. In other contexts, it may be possible to request raw data directly from authors, but this was not feasible within this project. The selection process is documented in EXCELFILE ECO_1 StudySelectionForEcoExposure.xlsx. A narrative summary of the included studies is provided below and followed by a graphical summary of the compiled data which is itself provided in EXCELFILE ECO_3 MeasuredCongenerLevels.xlsx. A graphical summary is provided because these large amounts of exposure data are not well suited to a narrative review.

5.2.2.2.2.1 Technical issues

The following simplifications were used for data entry:

- if only a range was given, the midpoint was used
- if two congeners were measured together, the joint value was used for both
- if value is given as less than x, x was used
- detection rate was not recorded

Each of these factors may make these estimates conservative for each congener but the loss in conservatism from not recording the full complement of PBDEs is an unknown; it is therefore not known whether these joint exposure estimates are conservative or not.

Table 13: Grouping of environmental exposure data

Group name	Database entries included in the group[a]	Number of identified articles
Environmental compartments		
Freshwater	water; water, river; water, lake; riverine runoff	22
Freshwater sediment	sediment, river; sediment, lake; sediment, core; sand; suspended particulate matter	77
Marine sediment	sediment, marine	34
Marine water	water, seawater	4
Soil	soil; soil, topsoil; soil, farm; Soil, core; compost; digestate; porewater	41
STP[b]	water treatment influent; water treatment effluent; waste water treatment plant (WWTP); wastewater, sludge; sewage; sewage sludge	28
Organism groups		
Aquatic plant	algae	2
Fish	fish	46
Invertebrate	bivalve; crustacean; gastropod; insect; invertebrates; starfish; worm	32
Microorganisms	bacteria; microbe; plankton	7
Terrestrial plant[c]	plant; vegetation; leaf; vegetation leaves; tree bark	11
Vertebrate[d]	amphibian (1); bird (52); mammal (9); reptile (1); rodent (2); sea mammal (5)	60

[a] text in this column indicates the database entries that were included in the group; the database entries for each article are provided in Appendix A. The level of detail given varied according to the original authors and the level of detail provided in the abstract, for example one author might describe a sample specifically as 'lake water' whilst another might describe a similar sample as only 'water' or fresh water.

[b] sewage treatment plant (STP).

[c] terrestrial plants could be considered as either an environmental compartment (8 database entries) or an organism groups (12 database entries), and they are placed here as an organism group since the available exposure data will necessarily include the consequence of biotransformation by plants.

[d] values in brackets for vertebrate grouping are number of articles per sub-group.

5.2.2.2.2.2 PBDE levels in environmental compartments, selected studies

Freshwater

Systematic review identified 22 studies. 3 were considered on the basis of date and number of congeners; data from one study was compiled for possible use in MRA (Zhang et al. 2010a).

Freshwater sediment

Systematic review identified 77 studies for this compartment, the most for any of the compartments considered. The 3 most recent studies with the highest number of congeners were selected, and one additional study was compiled for another grouping and included freshwater sediment data. 2 studies provided congener level data that could be compiled; both studied reported examining 46 congeners in samples from China (Zhang et al. 2010a) and the United States (Marvin et al. 2013).

(Marvin et al. 2013) reported data from 2001 and 2006 for freshwater sediment in the Detroit River, United States, and compared their observations to Canadian Federal Environmental Quality Guidelines of 44 ng/g for BDE 28, 39 ng/g for BDE 47, 0.4 ng/g for BDE 99 and BDE 100, 440 ng/g for BDE 153, 6700 ng/g for total octa BDE and 19 ng/g for BDE 209. Marvin et al found that BDEs 99, 100 and 209 frequently exceeded quality guidelines (described as levels below which there is judged to be little probability of adverse impacts on aquatic life). BDEs 28, 47 and 153 did not exceed guidelines, except for one sample for BDE-47 from 2006.

Marine water

Systematic search identified 4 studies of possible relevance and 2 recent studies that both examined 10 congeners were compiled (Moeller et al. 2011, Moeller et al. 2012). In 18 seawater samples obtained during an polar expedition cruise from the East China Sea to the Arctic, 10 PBDEs were analyzed in both the dissolved and particulate phases (Moeller et al. 2011). In the dissolved phase, BDEs 47, 99 and 100 were not detected in all samples and were present only at low levels (0.004 to 0.59 pg/L); BDEs 66 and 209 were only detected in one out of 18 samples at 0.17 and 0.18 pg/L; and BDEs 28, 85, 153, 154 and 183 were not detected in any samples. BDE-209 was detected more often in the particulate phase (4 of 14 samples) but at similarly low levels (0.1 to 0.17 pg/L); the converse was observed for BDEs 47, 99 and 100 in that they were less commonly detected in the particulate phase than the dissolved phase. In seawater from the North Sea, BDEs 47, 99, 100, 153 and 183 were detected at levels below 1pg/L, and BDE-209 at 1-5pg/L, whilst BDEs 28, 66, 85, 154 were undetectable (Moeller et al. 2012).

A very recent study that was published as this report was being finalised is now reviewed briefly here but could not be included in our systematic data compilation. Lohmann et al. analysed 10 PBDEs (BDE-28 -47, -66, -85, -99, -100, -153, -154, -183, -209) in water and air samples on an east-west transept across the tropical Atlantic Ocean in 2009 (Lohmann et al 2013). They reported that typical particle-bound concentrations of PBDEs in the surface water were low, at <1 pg/L, and that truly dissolved PBDE concentrations were lower, approximately 0.5pg/L for BDE-47 and less than 0.1pg/L for the other congeners, including BDE-209 which made only a minor contribution to the PBDE profile in water. Conversely, in air samples BDE-209 was the dominant congener and was detected at mean concentrations of 38 pg/m^3 (Southern Hemisphere) and 4 pg/m^3 (Northern hemisphere); the other PBDEs were present at concentrations (values for both hemispheres) of 3pg/m^3 (BDE-47), 2pg/m^3 (BDE-99) and < 1pg/m^3 (all other congeners). These authors found that their own research vessel was a significant source of PBDE contamination to the extent that results from active sampling did not provide a reliable indication of environmental PBDE levels; they were able to obtain valid environmental samples by using passive sampling with polyethylene sheet samplers (Lohmann et al. 2013).

Marine sediment

Systematic search identified 34 studies of possible relevance: three recent studies (2011, 2009, 2008) were selected on the basis of number of congeners studied (24, 46, 64). Oram et al. could not be used as they only provided levels for 2 congeners, despite reporting the study of 46 (Oram et al. 2008) Data was compiled for 17 sites around a Canadian wastewater outfall (deBruyn, Meloche, & Lowe 2009) and for a study of 24 congeners from China (Yu et al. 2011a).

Soil

Systematic review identified 41 studies from the following geographical regions: China (28 studies), United States (3), Japan (1), Taiwan (1), Kuwait (1), Spain (1), Sweden (1), Switzerland (1), Turkey (1), Canada (1), Australia (1), not region specific (2). 4 of these studies were published in the period 2010 onwards and had examined 40 or more congeners. Data from three studies were compiled for possible use in MRA (Jiang et al. 2010;Li et al. 2011;Zhang et al. 2010b). One additional study was also compiled for the terrestrial plants compartment and contained information on soil samples (Yang et al. 2008).

Zhang et al targeted 46 congeners and reported levels of 14 congeners in pond environments (soil, water, sediment) and in 6 fish species from South China (Zhang et al. 2010a). Li et al. developed a method to analyse 41 PBDEs in soil, and reported levels of 19 congeners in soil from an e-waste recycling site at Guiyu, China (Li et al. 2011), and Jiang et al. detected 29 out of 44 congeners in soil from urban Shanghai (Jiang et al. 2010).

Sewage treatment plant (STP)

Systematic review identified 28 studies that examined various stages through sewage treatment, including influent, effluent and sewage sludge. 20 studies measured PBDE levels in sewage sludge, and were considered for compilation for MRA. Only 2 studies had examined more than 40 congeners (North 2004;Oram et al. 2008) and neither were recent; 10 studies examined between 27 and 10 congeners; and 7 studies examined fewer than 10 congeners. 3 recent studies were considered in detail and data from 2 studies, from Korea (Hwang et al. 2012) and China (Yang et al. 2011), were compiled for possible use in MRA. Yang et al. reported data for only 5 out of 18 congeners targeted and they included a review of the same congeners in different geographical locations and at different times; this was reviewed in the section on temporal trends, see above.

5.2.2.2.2.3 PBDE levels in biota (including after biotransformation), selected studies

Aquatic plant

Systematic review identified 2 papers of possible relevance at aquatic plants, however only one of the papers was found to be relevant when the publication was read in detail. Yu et al. reported levels of 23 congeners in phytoplankton/algae and seaweed/macroalgae in estuarine bays in South China (Yu et al. 2011a). The dominant congener was BDE-209 in both organisms. Data was compiled for possible use in MRA.

Microorganisms

This grouping included microbes, bacteria and plankton. Systematic review identified 7 papers of possible relevance: 4 had relevance on consideration of full text and data was compiled from all 4 studies for possible use in MRA (Bartrons, Grimalt, & Catalan 2011;Yu et al. 2011a) (Hu et al. 2010) (Kuo et al. 2010). Unfortunately, Hu et al. provided quantitative information for other congeners but were only able to report for BDE-209 that the congener was detectable in 50 of biota samples, no quantitative information was available.

Fish

Systematic review identified 46 studies for fish. 3 studies were selected for further review based on date and number of congeners studied: 46 (Zhang et al. 2010a), 42 (Qin et al. 2009) and 19 (Carlsson et al. 2011).

Invertebrates

Systematic review identified 32 papers for invertebrates such as crustaceans, insects and worms. 2 studies examined 46 or 40 congeners, then the next largest studies examined around 20 congeners, one of which was very recent (2012) and was included for further review. Studies examined 46 congeners in invertebrates from Canada (deBruyn, Meloche, & Lowe 2009), 40 congeners in sample from Italy (Vigano et al. 2009) and 20 congeners in invertebrate samples from the United States (La Guardia et al. 2012).

Terrestrial plants

Systematic review identified 11 studies of plant leaves and tree bark. Few of the studies had examined large number of congeners: 7 examined fewer than 10, and the others studied 40, 23, 13 and 11 congeners. These latter studies were selected for further review, however they are not very recent dating from 2008 or earlier - with the exception of one 2012 study. Most recent studies had reported examining fewer than 10 congeners. Only three of the studies provided congener level data: a study of 40 congeners in leaf samples (Yang et al. 2008), a study of 13 congeners in tree bark (Zhu & Hites 2006) and a study of 11 congeners in samples of grass, haricot beans and saline seepweed (Jin et al. 2008). Yang et al. noted that PBDE levels in leaves are considered to reflect exposure through air, not transport from roots (Yang et al. 2008).

Vertebrates

Systematic review identified 60 studies for vertebrates including amphibians, birds, mammals, sea mammals, reptiles and rodents; fish were considered separately (see above) and human data is not included in this section. The most studied sub-groups within the vertebrates group were birds, and then mammals. The number of congeners examined was: >40 (4 studies), 30-40 (3 studies), 20-30 (7 studies), 10-20 (28 studies) and <10 (18 studies).

Eight studies were selected for further review in order to provide representation of each of the subgroups, and data from all studies was compiled for possible use in MRA. Reflecting the amount of data available, around half of the studies reported levels in birds. Studies of congener levels in birds included: a study of 49 congeners in the short-tailed shearwater (Tanaka et al. 2013); 46 congeners in ring-billed gull (Gentes et al. 2012); and 19 congeners in common eider (egg samples) and herring gull (egg and liver samples)(Carlsson et al. 2011). In addition, Yu et al reported levels of 16 congeners in the common kestrel and in 4 prey species including one mammal (rat), bird (sparrow), and insect(grasshopper and dragonfly) (Yu et al. 2011b).

Mammalian exposure is represented by a study of 12 congeners in polar bears and gulls (Verreault et al. 2005), and a study of 41 congeners in harbour seals, which was compiled as an example of exposure levels in a sea mammal(Shaw et al. 2008). Liu et al examined 8 congeners in amphibians (frogs) (Liu et al. 2011) and Hu et al reported levels of 10 congeners for a range of organisms; data for reptiles (turtles) was compiled here, and data for zooplankton was compiled elsewhere (see section on microorganisms) (Hu et al. 2010).

5.2.2.2.2.4 Identification of studies for possible use in MRA

The minimal outcome of the focused review was envisaged to be 36 exposure scenarios (3 per compartment or organism group, if available), however 'opportunistic' collection of data from studies being compiled for other compartments, organisms, time periods, or locations resulted in 144 scenarios being compiled, a large number of which can be considered superfluous to immediate use in environmental MRA (for the purpose of this project), see Table 14.

Removing these additional studies leaves 76 scenarios across the 12 groups, and these results will now be summarised.

Table 14: Summary of scenarios that are available in the data compilation but were not used for environmental MRA[a]

Citation (study that provided additional scenarios not used for MRA)	Number of scenarios that were removed	Reason for removing	Exclusion code[b]
(Hu et al. 2010)	2	Scenarios were not quantitative for BDE-209; BDE-209 was only reported as 'detected' and was not quantified	HU
(deBruyn, Meloche, & Lowe 2009)	32	Scenarios provided additional sampling of area around an outfall, for 2 species;, only the scenarios closest to the outfall was considered for MRA	X
(Yang et al. 2011)	32	Scenarios covered temporal and geographical trends. Data were only for 5 congeners, but article provides a literature review over multiple countries and years for sewage sludge. Data discussed in section on *Temporal trends*, see above	5
(Zhang et al. 2010a)	2	Scenarios were for fish feed, which was not selected as one of the environmental compartments for MRA	X

[a] this table lists the additional scenarios that were compiled when data was being extracted from published studies, but that was not directly relevant to environmental MRA; for example if a study included additional sampling sites around a primary chemical source, or reported temporal trends. This information is provided in the data compilation "EXCELFILE ECO_3" but was not used in environmental MRA (Section 6.3), where the aim was to survey a range of environmental media and organisms rather than to explore geographical or temporal effects/trends in detail.

[b] this code is also given in the second column ("ExclusionCode") of the data compilation "EXCELFILE ECO_3" where it can be used to filter the data to show the scenarios considered for use in environmental MRA.

5.2.2.2.2.5 Tabular and graphical summary

All results

Table 15 presents summary metrics for exposure data for all 76 scenarios. 70 congeners were measured in one or more scenarios, and the number of congeners targeted per study ranged from 6 to 49. The number of congeners actually measured (i.e. which were above the non-detect limit of the method) ranged from 3 to 46. The congener responsible for the highest measured value was determined for each scenario and was one of 4 congeners: BDE-209 in 43 scenarios; BDE-47 in 23 scenarios; BDE-99 in 9 scenarios and BDE-66 in one scenario (Table 15). The contribution made by the dominant congener ranged from 20 to 99.7% of the total PBDE level.

To provide a graphical overview of the percentage contribution of congeners within each scenario, Figure 6 shows stacked bar charts of the percentage contribution of each congener to the total PBDE level (sum of all measured in the scenario, on weight basis). The graphs shows stacked bar charts for each scenario, each stack contains all 70 congeners measured in any of the scenarios, sized according to their percentage contribution (by weight).

Evaluation of exposure data relevant to MRA approaches based on EQS-mandated monitoring for 6 congeners

MRA approaches based on the European Environmental Quality Standards (EQS) are potentially useful in low/early tier MRA (see section 6.3); therefore we examined whether studies have measured the 6 congeners (BDE-28, -47, -99, -100, -153, -154, hereafter referred to as "EQS congeners") mandated for calculation of the EQS (EU Directive 2013/39/EU), and the extent to which this subset of congeners captured the sum of PBDEs present. It is immediately noteworthy that the 'EQS congeners' do not include BDE-209. Table 15 includes the metrics calculated. 73 studies measured all 6 EQS congeners and 3 studies measured 5 of them. Not all of the scenarios are relevant in the context of the EQS, so it is clear that data on the EQS congeners is almost always available.

For scenarios in fish, the 6 EQS congeners constituted from 52 to 87% of the total sum of PBDEs (sum of all PBDEs measured in the scenario, on the basis of weight), indicating that only considering the EQS congeners could neglect from 23 to 48% of the PBDEs present. An interesting variation can be seen in loach; Loach were sampled in 4 scenarios, two for control sites and two for sites near e-waste recycling facilities. The EQS congeners constituted 87% of total PBDEs at control sites but only 52% at contaminated sites. This indicates that the contamination is with PBDE congeners not included in the 6 congeners specified for monitoring under the EQS. In these scenarios BDE-209 was undetectable at control sites and very low at the contaminated sites: so in this case the deficit in using EQS is not due to BDE-209 but to other congeners. In other compartments, EQS congeners constituted 1.5 to 22% (fresh water); 0.3 to 32% (fresh water sediment), 10 to 61% (marine water) and 0.3 to 46% (marine sediment) of the total PBDE level.

In many of the scenarios in which the EQS congeners did not constitute the majority of the total PBDE level, it could be seen (Table 15) that BDE-209 explained much of the difference between the total PBDE level and the amount covered by the EQS congeners. On average the EQS congeners accounted for 44% (mean of all scenarios) of the total PBDE level whilst the sum of EQS congeners and BDE-209 accounted for 83%. It is important to note that, when toxic potency is factored in, the percentage of the total PBDE load explained by a given congener may be less relevant, for example a congener that is highly toxic does not need to be present as a high proportion of the total PBDE level in order to contribute significantly to overall mixture toxicity. Finally, we note that, although it is normal practice in ecotoxicology to express chemical exposure on a weight basis, and we have conformed to that in this compilation, this practice may influence the comparison of chemicals that can vary greatly in molecular weight. For example the molecular weight of PBDE congeners vary around 4-fold, from 249.1 (monoBDEs) to 959.2 (BDE-209). BDE-47, the congener that dominated exposure measurements most after BDE-209 (see above), has a molecular weight of 485.5, around half that of BDE-209.

Table 15: Summary metrics for congener measurements

MAJOR GROUP/Subgroup	DECA DB ID#	Count	Sum (weight basis)						Sum as % of sum (All cong.)						Maximal contributor		
		No of cong. detected / no. targeted	All cong.	All except BDE-209	6 EQS cong.	All except 6 EQS cong.	All except BDE-209 and EQS cong.	Units	All except BDE-209	6 EQS congeners	All except 6 EQS cong.	All except BDE-209 and EQS cong.	BDE-209	EQS cong. and BDE-209	Highest measurement	Cong. responsible	% contribution of cong. to total
1. AQUATIC PLANTS																	
Sea weed/macroalgae	833	20/23	4.0	0.5	0.3	3.7	0.2	ng/g dw	12.9	7.5	92.5	5.4	87.1	94.6	3.5	-209	87.1
2. FISH																	
Loach	1054	10/39	349.7	349.7	301.8	47.9	47.9	ng/g lw	100.0	86.3	13.7	13.7	nd	86.3	141.9	-47	40.6
Loach	1054	27/39	19875.9	19874.5	10376.4	9499.5	9498.1	ng/g lw	100.0	52.2	47.8	47.8	0.0	52.2	6673.8	-47	33.6
herring and sprat	807	5/11	1.3	1.3	1.1	0.2	0.2	ng/g ww	100.0	85.6	14.4	14.4	Nd	85.6	0.6	-47	47.2
Loach	1054	8/39	1.1	1.1	1.0	0.2	0.2	ng/g ww	100.0	86.6	13.4	13.4	Nd	86.6	0.5	-47	41.1
Loach	1054	26/39	312.3	312.2	163.0	149.2	149.2	ng/g ww	100.0	52.2	47.8	47.8	0.0	52.2	104.9	-47	33.6
Bighead Carp	893	14/14	20586.0	17772.0	11707.0	8879.0	6065.0	pg/g lw	86.3	56.9	43.1	29.5	13.7	70.5	7908.0	-47	38.4
Bighead Carp	893	14/14	13198.0	12078.0	8950.0	4248.0	3128.0	pg/g lw	91.5	67.8	32.2	23.7	8.5	76.3	5877.0	-47	44.5
Bluntsnout Bream	893	13/14	10917.0	10054.0	6687.0	4230.0	3367.0	pg/g lw	92.1	61.3	38.7	30.8	7.9	69.2	3336.0	-47	30.6
Common Mullet	893	13/14	5856.0	5297.0	4161.0	1695.0	1136.0	pg/g lw	90.5	71.1	28.9	19.4	9.5	80.6	2676.0	-47	45.7
Crucian carp	893	14/14	30349.0	27587.0	21144.0	9205.0	6443.0	pg/g lw	90.9	69.7	30.3	21.2	9.1	78.8	16512.0	-47	54.4
Grass Carp	893	10/14	6042.0	6042.0	4907.0	1135.0	1135.0	pg/g lw	100.0	81.2	18.8	18.8	nd	81.2	3417.0	-47	56.6
Grass Carp	893	10/14	6159.0	6159.0	5031.0	1128.0	1128.0	pg/g lw	100.0	81.7	18.3	18.3	nd	81.7	3523.0	-47	57.2
Largemouth Bass	893	10/14	29858.0	29858.0	23625.0	6233.0	6233.0	pg/g lw	100.0	79.1	20.9	20.9	nd	79.1	16539.0	-47	55.4
Mud Carp	893	12/14	11848.0	11050.0	8865.0	2983.0	2185.0	pg/g lw	93.3	74.8	25.2	18.4	6.7	81.6	6356.0	-47	53.6
Mud Carp	893	12/14	14259.0	13516.0	11320.0	2939.0	2196.0	pg/g lw	94.8	79.4	20.6	15.4	5.2	84.6	7889.0	-47	55.3
Tilapia	893	14/14	73832.0	62708.0	47389.0	26443.0	15319.0	pg/g lw	84.9	64.2	35.8	20.7	15.1	79.3	25457.0	-47	34.5
Tilapia	893	12/14	13674.0	12712.0	10079.0	3595.0	2633.0	pg/g lw	93.0	73.7	26.3	19.3	7.0	80.7	5331.0	-47	39.0
3. FRESHWATER																	
Water (dissolved)	893	11/14	91.4	24.4	17.7	73.7	6.7	pg/L	26.7	19.4	80.6	7.3	73.3	92.7	67.0	-209	73.3
Water (dissolved)	893	11/14	74.1	22.1	16.1	58.0	6.0	pg/L	29.8	21.7	78.3	8.1	70.2	91.9	52.0	-209	70.2
Water (particle)	893	14/14	6433.2	729.2	99.4	6333.8	629.8	pg/L	11.3	1.5	98.5	9.8	88.7	90.2	5704.0	-209	88.7
Water (particle)	893	14/14	4812.2	885.2	200.4	4611.8	684.8	pg/L	18.4	4.2	95.8	14.2	81.6	85.8	3927.0	-209	81.6
4. FRESHWATER SEDIMENT																	
sediment, river	517	46/46	46510.5	19110.5	13614.8	32895.7	5495.7	pg/g	41.1	29.3	70.7	11.8	58.9	88.2	27400.0	-209	58.9
sediment, river	517	46/46	107206.9	48106.9	34656.0	72550.9	13450.9	pg/g	44.9	32.3	67.7	12.5	55.1	87.5	59100.0	-209	55.1
Sediment	893	14/14	17316.7	1238.7	56.0	17260.7	1182.7	pg/g dw	7.2	0.3	99.7	6.8	92.8	93.2	16078.0	-209	92.8
Sediment	893	14/14	9815.6	1289.6	77.4	9738.2	1212.2	pg/g dw	13.1	0.8	99.2	12.3	86.9	87.7	8526.0	-209	86.9

Table 15: Summary metrics for congener measurements

MAJOR GROUP/Subgroup	DECA DB ID#	Count	Sum (weight basis)						Sum as % of sum (All cong.)						Maximal contributor		
		No of cong. detected / no. targeted	All cong.	All except BDE-209	6 EQS cong.	All except 6 EQS cong.	All except BDE-209 and EQS cong.	Units	All except BDE-209	6 EQS congeners	All except 6 EQS cong.	All except BDE-209 and EQS cong.	BDE-209	EQS cong. and BDE-209	Highest measurement	Cong. responsible	% contribution of cong. to total
5. INVERTEBRATE																	
bivalve [a]	629	14/20	64870.0	21170.0	11856.0	53014.0	9314.0	ng/g lw	32.6	18.3	81.7	14.4	67.4	85.6	43700.0	-209	67.4
Caddisfly	1034	11/12	652.5	327.5	317.5	335.0	10.0	ng/g lw	50.2	48.7	51.3	1.5	49.8	98.5	325.0	-209	49.8
dragonfly	765	13/16	53.2	32.2	8.2	45.0	24.0	ng/g lw	60.5	15.4	84.6	45.1	39.5	54.9	21.0	-209	39.5
gammarid	1034	10/12	615.0	305.0	287.5	327.5	17.5	ng/g lw	49.6	46.7	53.3	2.8	50.4	97.2	310.0	-209	50.4
Gastropod [b]	629	12/20	47159.0	24459.0	22414.0	24745.0	2045.0	ng/g lw	51.9	47.5	52.5	4.3	48.1	95.7	22700.0	-209	48.1
grasshopper	765	13/16	70.6	30.6	1.9	68.7	28.7	ng/g lw	43.4	2.7	97.3	40.7	56.6	59.3	40.0	-209	56.6
mussel	1006	36/46	97283.9	91978.9	86090.5	11193.4	5888.4	pg/g dw	94.5	88.5	11.5	6.1	5.5	93.9	45100.0	-47	46.4
6. MARINE SEDIMENT																	
marine sediment	833	18/23	3.8	0.2	0.1	3.7	0.1	ng/g dw	4.8	1.5	98.5	3.3	95.2	96.7	3.6	-209	95.2
marine sediment	833	18/23	75.5	0.4	0.2	75.3	0.2	ng/g dw	0.6	0.3	99.7	0.3	99.4	99.7	75.1	-209	99.4
marine sediment	1006	34/46	6358.9	3808.9	2935.8	3423.1	873.1	pg/g dw	59.9	46.2	53.8	13.7	40.1	86.3	2550.0	-209	40.1
7. MARINE WATER																	
seawater	698	6/10	5.2	0.6	0.5	4.7	0.1	pg/L	11.3	9.6	90.4	1.7	88.7	98.3	4.7	-209	88.7
seawater	698	3/10	1.1	0.2	0.2	0.9	0.0	pg/L	21.7	21.7	78.3	0.0	78.3	100.0	0.9	-209	78.3
seawater	698	6/10	3.5	1.3	1.2	2.3	0.1	pg/L	38.0	35.2	64.8	2.9	62.0	97.1	2.2	-209	62.0
seawater	747	5/10	53.0	48.7	32.2	20.8	16.6	pg/L	92.0	60.8	39.2	31.3	8.0	68.8	16.6	-66	31.3
8. MICROORGANISMS																	
Algae/phytoplankton	833	23/23	48.3	4.9	1.8	46.5	3.1	ng/g dw	10.1	3.7	96.3	6.4	89.9	93.6	43.4	-209	89.9
microbial biofilm	758	15/15	0.6	0.4	0.1	0.5	0.3	ng/g OM	68.2	24.6	75.4	43.6	31.8	56.4	0.2	-209	31.8
microbial biofilm	758	15/15	21.2	3.2	2.1	19.1	1.1	ng/g OM	14.9	9.7	90.3	5.2	85.1	94.8	18.0	-209	85.1
plankton	909	6/6	30.1	0.1	0.1	30.0	0.0	ug/g lipid	0.3	0.3	99.7	0.0	99.7	100.0	30.0	-209	99.7
9. SOIL																	
soil	826	18/21	677.6	241.6	90.7	586.9	150.9	ng/g dw	35.7	13.4	86.6	22.3	64.3	77.7	436.0	-209	64.3
soil	1136	37/37	27161.1	23873.0	13138.6	14022.5	10734.4	ng/g dw	87.9	48.4	51.6	39.5	12.1	60.5	5469.4	-99	20.1
soil	886	30/30	754.6	277.6	87.2	667.4	190.4	ng/kg	36.8	11.6	88.4	25.2	63.2	74.8	477.0	-209	63.2
Soil	893	14/14	14575.2	1164.2	191.8	14383.4	972.4	pg/dw	8.0	1.3	98.7	6.7	92.0	93.3	13411.0	-209	92.0
Soil	893	14/14	7925.0	1224.0	364.2	7560.8	859.8	pg/dw	15.4	4.6	95.4	10.8	84.6	89.2	6701.0	-209	84.6
soil	765	16/16	4904.4	2104.4	205.4	4699.0	1899.0	pg/g dw	42.9	4.2	95.8	38.7	57.1	61.3	2800.0	-209	57.1

Table 15: Summary metrics for congener measurements

MAJOR GROUP/Subgroup	DECA DB ID#	Count	Sum (weight basis)						Sum as % of sum (All cong.)						Maximal contributor		
		No of cong. detected / no. targeted	All cong.	All except BDE-209	6 EQS cong.	All except 6 EQS cong.	All except BDE-209 and EQS cong.	Units	All except BDE-209	6 EQS congeners	All except 6 EQS cong.	All except BDE-209 and EQS cong.	BDE-209	EQS cong. and BDE-209	Highest measurement	Cong. responsible	% contribution of cong. to total
10. SEWAGE TREATMENT PLANT (STP)																	
sewage sludge	612	24/27	265.1	91.1	2.4	262.6	88.6	ng/g dw	34.4	0.9	99.1	33.4	65.6	66.6	174.0	-209	65.6
sewage sludge	612	27/27	306.8	119.8	21.3	285.5	98.5	ng/g dw	39.1	6.9	93.1	32.1	60.9	67.9	187.0	-209	60.9
11. TERRESTRIAL PLANT																	
grass	1137	11/11	1011.4	371.4	281.9	729.5	89.5	ng/g	36.7	27.9	72.1	8.8	63.3	91.2	640.0	-209	63.3
haricot bean	1137	10/11	157.9	67.9	51.6	106.3	16.3	ng/g	43.0	32.7	67.3	10.3	57.0	89.7	90.0	-209	57.0
saline seepweed	1137	4/11	70.7	18.7	0.0	70.7	18.7	ng/g	26.4	0.0	100.0	26.4	73.6	73.6	52.0	-209	73.6
leaves	1136	36/37	202.9	199.0	108.0	94.9	91.0	ng/g dw	98.1	53.2	46.8	44.8	1.9	55.2	43.0	-99	21.2
tree bark	1292	13/13	101.6	24.8	13.6	88.0	11.2	ng/g lw	24.4	13.4	86.6	11.0	75.6	89.0	76.8	-209	75.6
grass	765	10/16	5202.0	1802.0	201.0	5001.0	1601.0	pg/g dw	34.6	3.9	96.1	30.8	65.4	69.2	3400.0	-209	65.4
12. VERTEBRATE																	
BIRD [c]	765	15/16	359.9	262.9	65.9	294.0	197.0	ng/g lw	73.0	18.3	81.7	54.7	27.0	45.3	97.0	-209	27.0
BIRD [d]	765	16/16	244.9	176.9	27.3	217.6	149.6	ng/g lw	72.2	11.2	88.8	61.1	27.8	38.9	68.0	-209	27.8
BIRD [e]	519	31/49	66.5	30.3	1.8	64.7	28.5	ng/g lw	45.5	2.7	97.3	42.8	54.5	57.2	36.3	-209	54.5
RODENT, brown rat	765	16/16	138.1	93.1	10.5	127.6	82.6	ng/g lw	67.4	7.6	92.4	59.8	32.6	40.2	45.0	-209	32.6
SEA MAMMAL, seal	1069	25/25	2438.6	2437.4	2340.4	98.2	97.0	ng/g lw	100.0	96.0	4.0	4.0	0.0	96.0	1684.0	-47	69.1
AMPHIBIAN, frog	753	8/8	27.7	27.1	26.3	1.4	0.9	ng/g ww	97.9	94.9	5.1	3.1	2.1	96.9	9.9	-99	35.6
AMPHIBIAN, frog	753	8/8	17.1	15.9	15.7	1.5	0.3	ng/g ww	93.2	91.5	8.5	1.6	6.8	98.4	7.4	-99	43.3
AMPHIBIAN, frog	753	8/8	26.2	25.5	24.8	1.4	0.6	ng/g ww	97.3	94.8	5.2	2.5	2.7	97.5	10.9	-99	41.6
AMPHIBIAN, frog	753	8/8	28.4	26.4	25.6	2.8	0.8	ng/g ww	93.1	90.2	9.8	2.9	6.9	97.1	11.7	-99	41.1
AMPHIBIAN, frog	753	8/8	141.1	136.6	133.6	7.5	2.9	ng/g ww	96.8	94.7	5.3	2.1	3.2	97.9	67.1	-99	47.6
BIRD [f]	807	3/11	0.8	0.8	0.8	0.0	0.0	ng/g ww	100.0	100.0	0.0	0.0	nd	100.0	0.3	-99	42.0
BIRD [g]	1333	11/11	20.3	20.2	19.8	0.5	0.3	ng/g ww	99.4	97.7	2.3	1.6	0.6	98.4	8.8	-47	43.4
BIRD [g]	1333	11/11	20.0	19.8	19.5	0.5	0.3	ng/g ww	99.0	97.6	2.4	1.5	1.0	98.5	10.6	-47	53.1
BIRD [h]	807	8/11	8.4	7.1	6.4	2.0	0.7	ng/g ww	84.4	76.2	23.8	8.3	15.6	91.7	2.6	-47	30.9
BIRD [h]	807	5/11	7.2	7.2	7.2	0.0	0.0	ng/g ww	100.0	100.0	0.0	0.0	nd	100.0	2.7	-99	36.8
BIRD [i]	588	9/46	26.4	19.4	17.0	9.5	2.5	ng/g ww	73.5	64.2	35.8	9.3	26.5	90.7	7.0	-209	26.5
BIRD [i]	588	20/46	203.3	146.1	101.9	101.4	44.2	ng/g ww	71.9	50.1	49.9	21.7	28.1	78.3	57.2	-209	28.1
MAMMAL, polar bear	1333	11/11	5.8	5.7	5.6	0.2	0.1	ng/g ww	98.6	96.7	3.3	1.9	1.4	98.1	5.0	-47	86.5

Notes to Table 15**:**

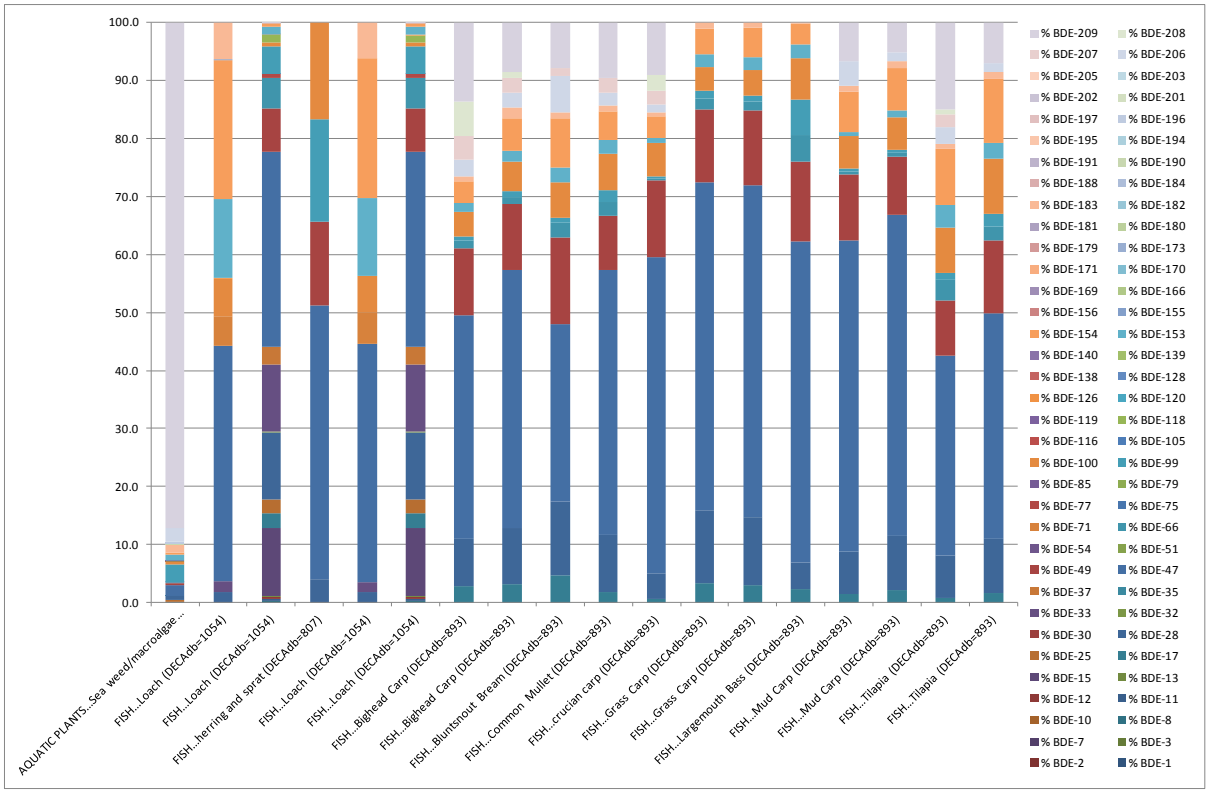
[a] Corbicula fluminea. [b] Elimia proxima. [c] Common kestrel. [d] Eurasian tree sparrow. [e] Short-tailed shearwater (Puffinus tenuirostris). [f] Common eider. [g] Glaucous gull.

[h] Herring gull. [i] Ring-billed gull.

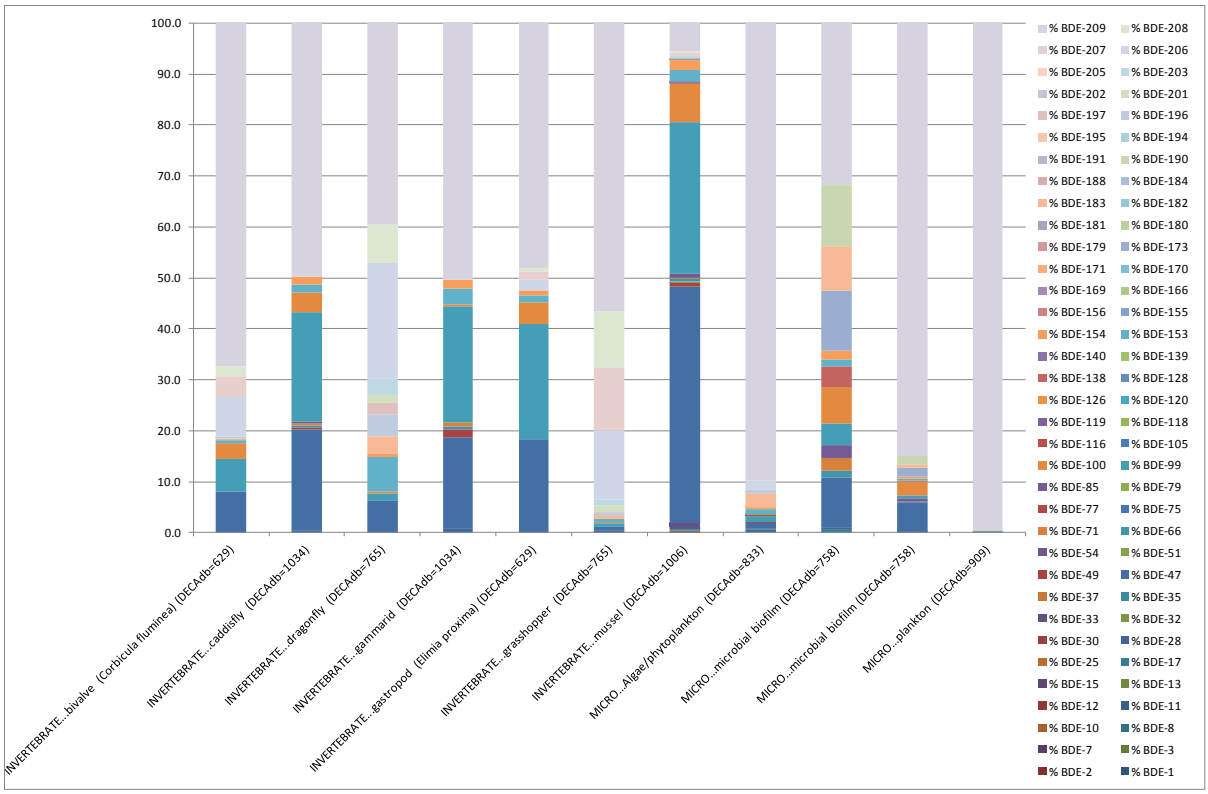
Abbreviations: Cong., congener.

Figure 6: Stacked bar charts showing the percentage contribution of each congener to the overall sum of PBDE weights

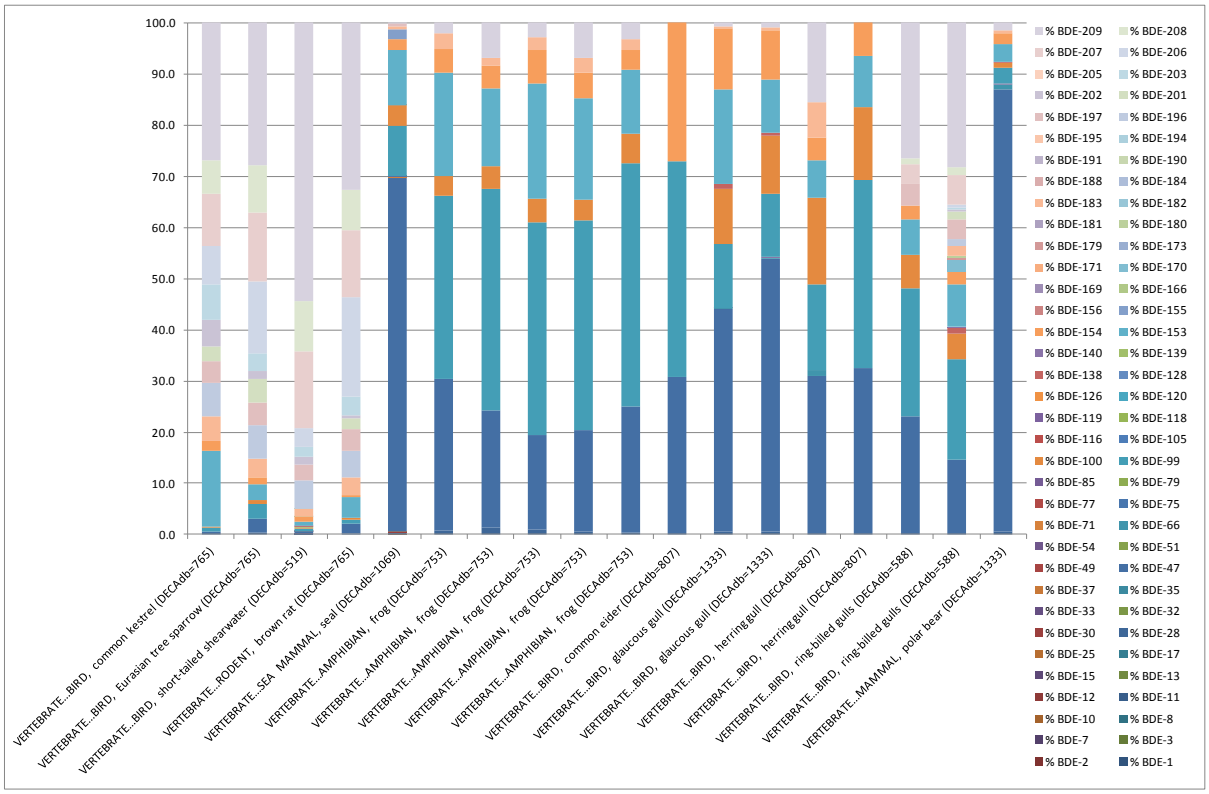
A. Aquatic plants and Fish



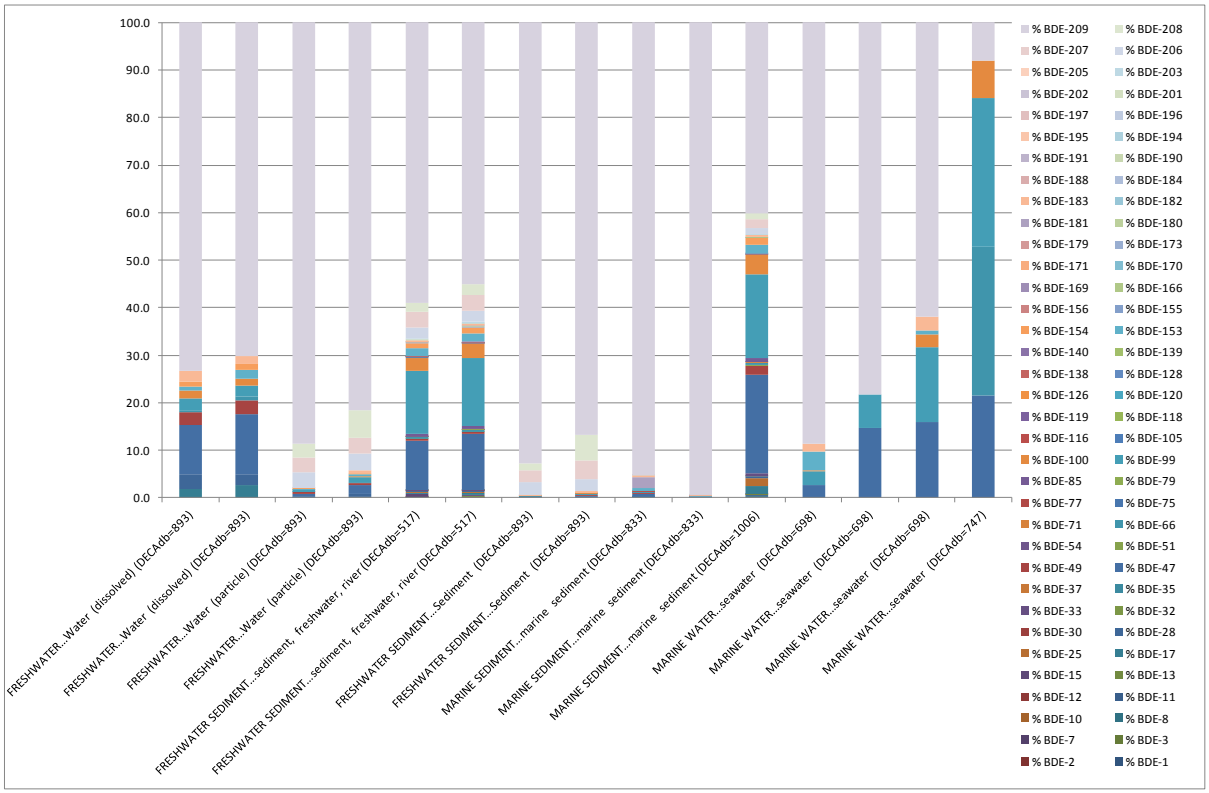
B. Microorganisms and Invertebrates



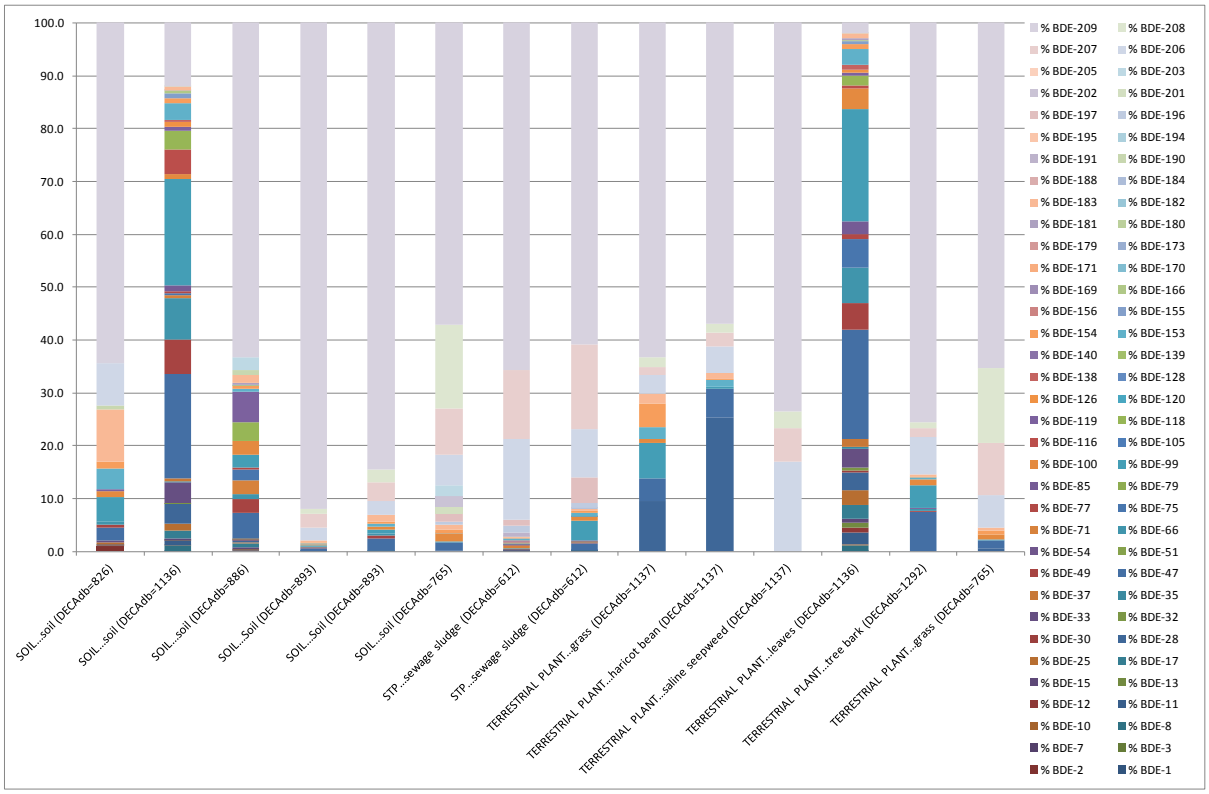
C. Vertebrates



D. Freshwater, Freshwater sediment, Marine water and Marine sediment



E. Soil, STP and Terrestrial plants



5.2.2.3 Conclusions

This section provided firstly, an overview of the PBDE congeners that were targeted in 304 studies (Section 5.2.2.2.1); and secondly, exposure levels for individual PBDE congeners in 12 environmental compartments and organism groups, focusing on recent studies that reported targeting high number of congeners (Section 5.2.2.2.2). A snapshot of environmental levels of PBDEs is provided by 76 different scenarios, which will be considered for use in environmental MRA (Section 6.3). In identifying scenarios, we favoured studies that had targeted the largest numbers of congeners because results from studies in which few congeners were targeted may always underestimate the extent of any mixture effect that may be present. At the same time, it must be noted that selecting studies on the basis of number of congeners may have penalised studies with higher quality requirements for stating a congener as a target, since they would have report fewer congeners as targets.

Data collection was identified as a major technical issue, with focused data collection being problematic and complicated by the fact that public literature databases do not allow for the direct retrieval of data on a particular PBDE congener. The issue is complicated by variable quality in the reporting of the PBDE congeners that were targeted, detected and quantified. Reporting total (sum) PBDE levels is common, but these levels are rendered incomparable by differences in the number of congeners that were targeted in each study.

5.2.2.4 References

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5.2.3 Possible toxic effects of PBDE mixtures in the environment

5.2.3.1 Literature selection

The aims of this review of the ecotoxicological literature are two-fold; the first objective is to describe and update the current state-of-knowledge of the potential effects of the single congener BDE-209 in environmental receptors, the second is to collate data on the effects of other PBDE congeners that co-occur with BDE-209 in the environment. Because knowledge of the effects of other PBDE congeners is required to carry out a mixture risk assessment, this second objective focuses specifically on the species or taxonomic group for which data is available for BDE-209.

This section considers experimental studies with both single congeners and commercial or synthetic mixtures. Studies of the correlation between levels of PBDE congeners in biota and population declines or other ecological effect are relevant but cannot be used for the purpose of predicting mixture toxicity and were therefore not considered here.

The European Union has published risk assessment reports for the three commercial mixtures pentaBDE, octaBDE and BDE-209 (European Chemical Bureau 2001; 2002; 2003). An update of the risk assessment for BDE-209 is available (European Chemical Bureau 2004). UNEP has also published risk profiles for the pentaBDE and octaBDE commercial mixtures (UNEP 2006; 2007). These reports formed the basis of this review. Information provided in the Norwegian nomination report on c-decaBDE to the Stockholm Convention was also considered where relevant (UNEP 2013).

The following step was to update and complete the literature on the ecotoxicology of BDE-209. The Annex XV dossier for BDE-209 published recently by the European Chemical Agency contains such an update and this material was reviewed and summarised (ECHA 2012). The three studies included in the ecotoxicity section of the 2009 environmental risk evaluation carried out by the UK Environment Agency (Brooke et al. 2009) were also considered for inclusion. Further, the literature database contains 43 references relevant to the ecotoxicological effects of BDE-209. Abstracts were scanned for eligibility to complete the dataset for BDE-209. Twenty studies from the database were selected for inclusion.

In order to complete and update the dataset for the pentaBDE and octaBDE commercial mixtures as well as collate any data for single PBDE congeners for relevant species and/or taxonomic group, the ecotoxicological data considered in the dossier on the derivation of environmental quality standards for PBDEs (Rodriguez-Romero 2011) was checked for eligibility. Finally, additional literatures searches were carried out in Web of Science. The search algorithm combined the term 'BDE' with the desired species or taxonomic group, e.g. 'BDE' AND 'Amphibian'. Titles were then scanned and relevant articles were selected. Further, relevant articles cited in the literature reviewed were also added.

Information that may be required for a mixture risk assessment was collated. This includes a critical effect dose such as an EC_x or LC_x when derived, or the highest concentration tested where no significant effects were observed for the most sensitive relevant endpoint. The composition of the mixture or purity of the compound tested was also noted when reported.

As the purpose of this review is to investigate the feasibility of carrying out an ecotoxicological mixture risk assessment, emphasis was primarily on apical endpoints assumed to affect populations, such as growth, reproduction and survival. Studies reporting effects that may be informative in terms of a putative toxic mode of action were also considered. When more than one study was available in the same species for the same endpoints, studies in which only one concentration had been tested were not considered further.

With regards to the quality of the selected studies, particular attention was given to the biological relevance of the route of exposure and whether concentrations of test compounds had been verified analytically.

5.2.3.2 Results

5.2.3.2.1 Aquatic environment – pelagic organisms

5.2.3.2.1.1 Algae

The European risk assessment reported for BDE-209 detailed one study that had investigated growth inhibition in two marine species of algae and one freshwater species. BDE-209 reduced growth of all three species by less than 50% at the highest concentration tested, the limit of solubility. It was therefore concluded that it was not possible to derive an EC_{50}. A recent toxicological profile drafted by the Japanese Ministry of the Environment stated that the growth inhibition EC_{50} for another species of freshwater algae (*Selenastrum capricornatum*) would be in excess of 5.2 µg/l but gives no detail of the experimental protocol (Ministry of the Environment 2012). The database contained one additional recent study that had derived growth inhibition EC_{50} in two marine algal species but these where well in excess of the solubility limit of BDE-209 (Zhang et al. 2013a).

In the European risk assessment reports, there was one study of the toxicity to a species of freshwater algae available for the pentaBDE mixture but none for octaBDE (European Chemical Bureau 2001; 2003). They reported an EC_{10} around 3 µg/l. For the derivation of EQS for PBDEs under the European Water Framework Directive, an EC_{50} of 70 µg/l (24h-NOEL = 6.6 µg/l) from a more recent study by Källqvist et al. (2006) of growth inhibition of the marine algae *Skeletonema costatum* by BDE-47 was also considered. The literature search yielded another two studies for specific congeners for species in which BDE-209 had been tested, namely BDE-99 in *Selenastrum capricornatum* and BDE-47 in *Karenia mikimotoi*. Both reported EC_{50}s greater than 200 µg/l, well in excess of water solubility (Evandri et al. 2003; Zhang et al. 2013b). Additionally, Mhadhbi et al. (2012a) had tested the same congeners and BDE-154 in another marine species of algae (*Isochrysis galbana*). The latter species appears to be more sensitive and 72h-EC_{50}s of 26, 30 and 244 µg/l were derived for BDE-47, -99 and -154, respectively.

In summary, there seem to be noticeable differences in sensitivity between algal species. On the basis of these data, it would appear that BDE-209 is not toxic to either marine or freshwater algal species. Toxicity to algae of other PBDE congeners appears to decrease as the number of bromine atoms increases.

5.2.3.2.1.2 Invertebrates

5.2.3.2.1.2.1 Daphnia magna (freshwater)

There were no experimental data available for *D. Magna* in the European risk assessment report for BDE-209. The literature search identified one recent study which reported that no significant effect on survival or molting could be seen at the highest concentrations tested (4.55 and 500 µg/l respectively)(Davies & Zou 2012). This is in agreement with the report of the Japanese Ministry of the Environment (2012).

Davies and Zou (2012) had also tested BDE-28, -47, -99 and -100 in *D. magna*. BDE-99 was the most acutely toxic with an LC_{50} of 2.6 µg/l whilst toxic effects on molting appear to increase as the number of bromine atom decrease. In fact no effect on molting could be seen for BDE-99 and -100 at sub-lethal concentrations in the low µg/l range. These values are in good agreement with the chronic NOEC reported in the European risk assessment report for pentaBDE (European Chemical Bureau 2001). However, Evandri et al. (2003) found a value one order of magnitude greater for the acute lethal effects of BDE-99 and the study by Källqvist et al. (2006) derived a chronic NOEL that was considered for the derivation of an PBDE EQS about twice as high as those derived for acute effects for BDE-47 by Davies and Zou (2012).

5.2.3.2.1.2.2 Brackish and marine crustacean species

There were no studies of BDE-209 with marine species. The literature search did however identify a study where a number of PBDE congeners (-28, -47, -99, -100) had been tested in a marine copepod (*Arcatia tonsa*) (Wollenberger et al. 2005). Effects on larval development occurred at lower concentrations with higher brominated congeners in the marine copepod.

Two studies investigated both acute lethality and larval development of several PBDE congeners including BDE-209 in the brackish water copepod *N. Spinipes* (Breitholtz and Wollenberger 2003; Breitholtz et al. 2008). The higher brominated congeners BDE-183 and BDE-209 did not have any effect on either endpoints at concentrations up to 100 mg/l. For other congeners, BDE-24, -47, -99, -100, potency of effects on larval development was inversely related with bromination, lower brominated congeners had effects in the low µg/l. However this endpoint was not considered reliable for the derivation of an EQS for PBDEs (Rodriguez-Romero 2011). The same report mentioned the study by Key et al. (2008) that found effects of BDE-47 in the brackish water grass shrimp (*Palaemonetes pugio*) but did not consider it.

5.2.3.2.1.2.3 Mollusca

The database contains one study that had investigated DNA damage in the Zebra mussel (Riva et al. 2007). Although this is not an apical endpoint, it is of interest as oxidative stress has also been studied in fish and birds as a putative toxic mode-of-action for PBDEs (see sections 5.2.3.2.1.3.4 and 5.2.3.2.4.1). For BDE-209, Riva et al (2007) used a COMET assay and found a significant increase of DNA damages at 2 µg/l compared to controls, but not at the highest concentration tested. They attributed this lack of dose-effect relationship to the formation of less-brominated congeners. There were however no changes in the frequency of micronucleus in the corresponding assay.

The literature search revealed that the same group have also investigated DNA damage and oxidative stress in the Zebra mussel following exposure to BDE-47, -100 and -154. They found that those congeners were also able to induce some reversible DNA damage at concentrations of the same order of magnitude (Parolini & Binelli 2012; Parolini et al. 2012). The zebra mussel appears to be most sensitive to BDE-154 and no pattern of correlation between bromination and toxicity emerged from the available data.

From the data currently available, the picture that emerges in relation to the toxicity of PBDE congeners is one where BDE-209 is not toxic, whereas some of the lower brominated congeners may be moderately toxic. The contribution of BDE-209 in that context seems to be solely related to the fact that it acts as a long-term environmental sink for the lower brominated congeners.

5.2.3.2.1.3 Fish

5.2.3.2.1.3.1 Acute toxicity

The European risk assessment reports find that all three commercial PBDE mixtures, penta-, octa- and decaBDE are not acutely toxic to the orange-red killifish (*Oryzias latipes*) also known as the Japanese medaka (European Chemical Bureau 2001; 2002; 2003). The literature search strategy retrieved a further ten studies of acute toxicity in three fish species; namely the zebrafish (*Danio rerio*), the turbot (*Psetta maxima*) and the rainbow trout (*Oncorhynchus mykiss*) (Table 16).

The study of the acute toxicity of BDE-209 by Chen et al. (2012b) was mentioned in the Annex XV dossier for BDE-209 (ECHA 2012). This study also identified some effects on weight and thyroid parameters that were dismissed by ECHA as the concentration in water at which effects were seen was several orders of magnitude above the water solubility limit. A study of the acute toxicity of BDE-209 in zebrafish embryos following exposure to contaminated sediment demonstrated both the potential importance of this route of exposure and neurodevelopmental toxicity (Garcia-Reyero et al. 2013).

Generally, no effects on survival have been observed after short-term exposures at the embryo or larval stages in the zebrafish or the rainbow trout with any of the other PBDE congeners or commercial mixtures that have been tested. Some sublethal effect become significant particularly as the length of exposure is increased (Lema et al. 2007; Kuiper et al. 2008).

Investigations in the turbot were also considered when deriving an EQS for PBDEs (Rodriguez-Romero 2011) and it is clear that this species appears to be particularly sensitive to lower brominated PBDE congeners (Mhadhbi et al. 2010; Mhadhbi et al. 2012b).

These studies point to the possibility that guideline experimental protocols of acute toxicity may miss sublethal effects following exposure to more environmentally realistic routes.

5.2.3.2.1.3.2 Chronic apical effect

The European risk assessment report for decaBDE included two studies on fish. Kierkegaard et al. (1999) examined the uptake of BDE-209 in rainbow trout and reported increased liver weights at the dose tested (7.5-10 mg/kg body weight/day) but no significant changes in condition factor. Stapleton et al (2004) found a reduction in growth rate following dietary exposure to 940 ng/kg food in the common carp (Table 17).

Three additional studies were included in the Annex XV dossier (ECHA 2012) (Table 17). He et al. (2011) found no effect on growth in the F0 or F1 generations in a multi-generation study with the zebrafish after waterborne exposures up to 1 µM. They did however observe significant changes in gonadal development in the parent generation as well as behavioural and other developmental effects in the non-exposed F1 generation. Chen et al. (2012b) reported reduced weights in zebrafish exposed to 1.92 mg/l BDE-209 in the water. This test was considered invalid by ECHA given the fact that nominal concentrations were well above the water solubility limit. The study nonetheless qualitatively reflects that effects can occur at the water solubility limit. By contrast, Kuo et al. (2010) examined the effect of dietary exposure on otolith increment width in the lake whitefish at the highest exposure dose (2 µg/g diet) and found a statistically significant reduction in this group. This dietary dose is of the same order of magnitude as that used by Stapleton et al. (2004). Our literature search identified another study investigating changes in thyroid-related genes and evidence of thyroid disruption that had also found that the length of female rare minnows was decreased following waterborne exposure (10µg/l) to BDE-209 (Li et al. 2011) (Table 17).

There are therefore indications that BDE-209 can affect growth following dietary exposure at least in some species of fish. It is however difficult to derive a NOEL or critical effect dose as effects have been investigated in only one dose group.

The European risk assessment reports for other commercial mixtures (penta- and octaBDE) were also based on an effect on growth in the rainbow (Table 17). ECHA (2012) had also identified the study by Chen et al. (2012a), a multigenerational study with zebrafish that not only found effects on growth following waterborne exposure to DE-71 in the parent generation, but also effects on locomotor behaviour in the non-exposed F1 generation. The study by Timme-Laragy et al (2006) was cited in the report on the derivation of an EQS for PBDEs but not considered further. It reported an effect on hatching delay in the killifish following waterborne exposure to DE-71. Additionally, the literature search identified another two studies with commercial pentaBDE mixtures reporting reproductive effects on spawning success (Holm et al. 1993) and hatching rate (Yu et al. 2011).

Congener-specific (other than BDE-209) toxicity data was found only for BDE-47 in the regulatory literature (Muirhead et al. 2006 in Rodriguez-Romero 2011) and the peer-reviewed literature (Chou et al. 2010). The effects reported were related to reproduction and behaviour.

Effects on the immune system had not been investigated with BDE-209 but were reported in the salmon following exposure to a synthetic mixture of BDE congeners (Arkoosh et al. 2010) and the marine medaka following exposure to BDE-47 (Ye et al. 2011;Yu et al. 2013).

Table 16. Acute toxicity data for fish species

Test product	Exposure route	Species	Critical effect	Dose	Reference
BDE-209	Water	Zebrafish	Early life stage - Weight and survival	14d-NOEL = 380 µg/l	Chen et al, 2012b
	Sediment	Zebrafish	Early life stage – swimming behaviour	7d-NOEL < 13.67 mg/kg	Garcia-Reyero et al. 2013
DE-71 (c-pentaBDE)	Water	Zebrafish	Larval survival	30d-NOEL = 50 µg/l	Kuiper et al, 2008
BDE-47	Water	Zebrafish	Embryo-larvae mortality	96h-LC50 = 5,370 µg/l	Chan & Chan 2012
			Survival	96h-LC50 > 5,000 µg/l	Zheng et al. 2012
			delayed hatching, reduced growth, dorsal curvature	96h-NOEL = 100 µg/l	Lema et al, 2007
		Turbot	Embryo development	48h-LC50 = 24.07 µg/l	Mhadhbi et al, 2010
			Larval development	96h-NOEL = 0.49 µg/l	Mhadhbi et al, 2012
	Injection	Rainbow trout	Early life stage	LD50 > 12 µg/g egg	Hornung et al. 1996
BDE-49	Water	Zebrafish	Developmental and neurotoxicity	96h-NOEL < 2,000 µg/l	McClain et al. 2012
BDE-85	Injection	Rainbow trout	Early life stage	LD50 > 12 µg/g egg	Hornung et al, 1996
BDE-99	Water	Turbot	Embryo development	48h-LC50 = 30.89 µg/l	Mhadhbi et al, 2010
			Larval development	96h-NOEL = 1.61 µg/l	Mhadhbi et al, 2012
	Injection	Rainbow trout	Early life stage	LD50 > 12 µg/g egg	Hornung et al, 1996
BDE-154	Water	Turbot	Embryo development	48h-LC50 = 47.6 µg/l	Mhadhbi et al, 2010

Table 17. Chronic Toxicity data for apical endpoints in fish species

Test product	Exposure route	Species	Observed effects	Critical dose	Reference
BDE-209	Diet	Rainbow trout	Increased liver weight	NOEL < 7.5-10 mg/kg body weight/day	Kierkegaard et al. 1999
		Common carp	Growth rate reduction	NOEL < 940 ng/kg food	Stapleton et al. 2004
		Zebrafish	Growth (otolith increment widths)	30d-NOEC < 2 µg/g food	Kuo et al. 2010
	Water - parental transfer	Lake whitefish	Gonad development in F0, neurobehaviour and development in non exposed F1	150d-NOEL < 0.96 µg/l	He et al. 2011
c-pentaBDE	Water	Rainbow trout	Growth	87d-NOEL = 8.9 µg/l	European Chemical Bureau 2001
DE-71 (c-pentaBDE)	Water	Killifish	Hatching delay	NOEL = 1 µg/l	Timme-Laragy et al. 2006
	Water - parental transfer	Zebrafish	Growth (F0), Locomotor behaviour (F1)	150d-NOEL < 0.16 µg/l	Chen et al. 2012a
		Zebrafish	Hatching rate, Body weight (F1)	NOEL < 1 µg/l	Yu et al. 2011
Bromskal-7 (c-pentaBDE)	Diet	Three-spined stickleback	Spawning success	NOEL = 72 mg/g body weight (actual body burden)	Holm et al. 1993
BDE-47	Diet	Zebrafish	Locomotion, behaviour	NOEL = 81.3 ng/g food	Chou et al. 2010
		Fathead minnow	Egg production	25d-NOEL < 28.7 µg/pair/day	Muirhead et al. 2006

5.2.3.2.1.3.3 Thyroid disruption

As BDE-209 was highlighted as a potential thyroid disrupter in rodents, effects on thyroid hormone levels or thyroid-related genes have also been investigated in recent articles retrieved in the database. Because of the role of thyroid hormones in growth and development, particularly neurodevelopment, this is also of interest as a putative mechanism for apical effects.

The three studies of the effects of BDE-209 on thyroid endpoints have investigated three different species of fish exposed by three different routes; namely, Li et al (2011) exposed Chinese rare minnows via the water, rainbow trout were injected peritoneally (Feng et al. 2012), and finally Noyes et al. (2013) exposed fathead minnows via the diet (Table 18). All three studies report effects on thyroid hormones or thyroid-related genes, but it is difficult to compare the dosages used directly. With dietary exposure, effects were already seen in the low dose group (0.41 ng/g wet weight of food) (Noyes et al. 2013). There was also some evidence of non-monotonic dose-responses for some endpoints at given time points and these were interpreted as consistent with compensatory mechanisms.

There is therefore consistent evidence that BDE-209 can have an effect on thyroid hormone levels in several species of fish.

Evidence that other congeners may also disrupt the hypothalamus-pituitary-thyroid axis is equivocal. Non significant changes in plasma thyroxine were seen in the flounder exposed to large doses of the commercial mixture pentaBDE DE-71via sediment and the diet (Kuiper et al. 2004) whilst no changes were recorded in zebrafish exposed to BDE-47 via the diet (Torres et al. 2013). By contrast, lake trouts exposed to a synthetic mixture of BDE congeners in food (including BDE-209) had lower levers of thyroxine T4 in the low and high dose groups compared to controls (Tomy et al. 2004). Lower T4 levels were maintained in the high dose group after 112 days depuration. Depressed T4 levels were also observed in fathead minnows exposed to BDE-47 via the diet (Lema et al. 2008). Unfortunately, neither study in fathead minnows reported the weight of the fish and it is therefore not possible to compare the potency of BDE-47 and BDE-209. Yu et al. (2011) recorded changes in thyroid hormone levels in a multi-generational study with the zebrafish in the parent generation, the F1 generation as well as in the eggs of the F0 generation. It should also be noted that some effects on thyroid hormones or thyroid-related genes were seen in acute toxicity studies with the zebrafish following waterborne exposure to BDE-47 (Chan & Chan 2012) or DE-71 (Kuiper et al. 2008) or in the non-exposed F1 generation (waterborne parental exposure) with DE-71 (Yu et al. 2011).

There is therefore evidence that other congeners may also act by disrupting thyroid hormones at least in some species of fish.

5.2.3.2.1.3.4 Oxidative stress

Another toxic mode-of-action that has attracted some scientific interest in relation with PBDE is oxidative stress. The database contained three studies of the effects of BDE-209 exposure on hepatic biomarkers of oxidative stress in the goldfish (Feng et al. 2013a; Feng et al. 2013b; Zhao et al. 2011). In the two studies that used intraperitoneal injection as the exposure route, effects were already seen in the lowest dose tested (10 mg/kg body weight), whilst in the study using waterborne exposure (up to 4 µM), effects were transient and appear to be reversible. It is therefore difficult to conclude on the role that oxidative stress may play in eliciting apical effects.

Our search of the literature identified one study that had measured biomarkers of oxidative stress in the goldfish following exposure to other BDE congeners. For both BDE-47 and BDE-99, effects were already seen at the lowest dose injected peritoneally (0.04 mg/kg) (Lu et al. 2013).

Table 18. Evidence for thyroid disruption in fish toxicity studies

Test product	Exposure route	Species	Critical effect dose	Reference
BDE-209	Diet	Fathead minnow	42d-NOEL <3 ng/g bw/day or 0.41 ng/g ww food	Noyes et al, 2013
	Intraperitoneal injection	Rainbow trout	21d-NOEL < 50 ng/g body weight	Feng et al, 2012
	Water	Chinese rare minnow	21d-NOEL = 1 µg/l	Li et al 2011
DE-71 (c-pentaBDE)	Sediment and food	European flounder	NOEL > 700 µg/g TOC + 14,000 µg/g lipid	Kuiper et al, 2008
	Water- parental transfer	Zebrafish	NOEL = 1 µg/l	Yu et al. 2011
Synthetic mixture*	Diet	Lake trout	56d-NOEL < 22.8 ng/g diet	Tomy et al, 2004
BDE-47	Diet	Zebrafish	120d-NOEL > 25 µg/g diet	Torres et al, 2013
	Diet	Fathead minnow	21d-NOEL < 2.38 µg/pair/day	Lema et al 2008

*5.7% BDE-28, 9.2% BDE-47, 7.5% BDE-66, 3.9% BDE-77, 6.6% BDE-85, 4.8% BDE-99, 4.8% BDE-100, 7% BDE-138, 10.1% BDE-153, 7.9% BDE-154, 9.2% BDE-183, 8.3% BDE-190, 14.9% BDE-209

5.2.3.2.1.4 Amphibians

Because BDE-209 and other PBDE congeners are suspected thyroid disrupters, a number of studies have investigated their effects on frog metamorphosis. The study by Qin et al. (2010) is mentioned in the Annex XV dossier for BDE-209 (ECHA 2012) but was disregarded by ECHA because it was not performed in accordance with test guidelines or good laboratory practice (GLP). They reported a significant delay in forelimb emergence in the highest tested dose of the technical mixture of BDE-209 DE-83R (1 µg/l).

The literature search identified three other studies that also reported an effect on amphibian metamorphosis following exposure to the commercial pentaBDE mixture DE-71 or the single congeners BDE-47 and BDE-99. Balch et al. (2006) also used the African clawed frog (*Xenopus laevis*). However, the animals were exposed to DE-71 via the diet and intraperitoneal injection was used in the experiment with BDE-47 and BDE-99. These data can therefore not be directly compared with the data for BDE-209. They show however that BDE-47 is more potent than BDE-99 as no effect was seen with BDE-99 at the highest tested dose (100 µg per tadpole). The leopard frog appeared to be particularly sensitive to dietary exposure to DE-71 (7 ng/g wet food) (Coyle & Karasov 2010) whereas effects were seen at concentrations above 1 mg/g food for both BDE-47 and BDE-99 in the Western Clawed frog (Carlsson et al. 2007).

5.2.3.2.2 Aquatic environment - benthic organisms

5.2.3.2.2.1 Invertebrates

The European risk assessment reports derived sediment PNECs on the basis of tests carried with the oligochaete *Lumbriculus variegates* (European Chemical Bureau 2001; 2002; 2003). In short, no effects were seen at concentrations up to 4,536 mg/kg (dry weight) or 3,841 mg/kg (dry weight) for sediment containing 2.4% or 5.9% organic carbon respectively for BDE-209. No effects were seen with octaBDE either however the highest concentrations tested were lower (1,340 and 1,272 mg/kg dw for sediments containing 2.4% and 5.9% organic carbon respectively). For the pentaBDE mixture, a NOEL of 3 mg/kg dry weight was determined. Additionally, pentaBDE had been tested in a crustacean and an insect.

No new studies were identified either in recent reports or in the peer-reviewed literature.

5.2.3.2.2.2 Microorganisms

Effect on activated sludge respiration inhibition were also reported in the European Risk Assessment Reports for BDE-209 and octaBDE (European Chemical Bureau 2002; 2003). For both commercial mixtures, no effects were seen at concentrations up to 15 mg/l. They would therefore not be expected to affect wastewater treatment.

The database identified one study that had considered the effect of BDE-209 on the composition of the bacterial community in sediment (Liu et al. 2011;Wu et al. 2013). They detected a significant increase in diversity at concentration above 10 mg/kg. The ecological significance of this effect and whether this impact is negative remains unclear.

5.2.3.2.3 Terrestrial environment

5.2.3.2.3.1 Micro-organisms

The European risk assessment report for BDE-209 does not contain any information on soil micro-organisms. The literature database contains four studies on the effects of BDE-209 on soil microbial communities. In short, effects of BDE-209 contamination included suppression of enzymatic activity, reduction in community diversity and a shift in community structure (Liu et al. 2011;Zhang et al. 2012b;Zhang et al. 2012a;Zhu et al. 2010). Effects were seen at 1 mg/kg dry weight and above. Additionally, Liu et al. (2011) observed the BDE-15 increased the bacterial count at concentrations above 1 mg/kg dry weight.

The pentaBDE European risk assessment report gives the results of a nitrogen production test with lucerne meal. No effects were observed up to the highest concentration tested 1 mg/kg dry weight.

5.2.3.2.3.2 Plants

The European risk assessment reports base their evaluation on standard phytotoxicity tests on monocots and dicots (European Chemical Bureau 2001; 2002; 2003).Results of those tests are summarised in Table 19 below. Only pentaBDE exhibited phytotoxicity.

Table 19. Phytotoxicity tests results used in the European risk assessment reports for the penta-, octa- and BDE-209 commercial mixtures

	pentaBDE	**octaBDE**	**BDE-209**
Monocots: Corn (*Zea mays*), onion (*Allium cepa*), ryegrass (*Lolium perenne*)	21d-EC_5 = 16 mg/kg dw	21d-NOEL > 1,190 mg/kg dw	21d-NOEL > 5,349 mg/kg dw
Dicots: Cucumber (*Cucumis sativa*), soybean (*Glycine max*), tomato (*Lycopersicon esculentum*)	21d-NOEL = 125 mg/kg dw	21d-NOEL > 1,190 mg/kg dw	21d-NOEL > 5,349 mg/kg dw

The database contained a recent study that had not been considered in previous assessments and it investigated oxidative stress as a mechanism of phytotoxicity. Reactive oxygen species are known to be involved in inducing tissue injury in plants exposed to pollutants. Antioxidant enzymes, including superoxide dismutase (SOD) and catalase (CAT) can be used to characterise oxidative stress response produced by contamination. In their test system, Xie et al (2013b) noted effects on effects on reactive oxygen species and antioxidant response at concentrations above 1 mg/kg (dry weight) although some responses appeared to be non-monotonic. Effects on apical endpoints such as pigment contents and root length effects were seen at 50 or 100 mg/kg dw (NOEL 10 mg/kg dw).

5.2.3.2.3.3 Earthworms

The European risk assessment reports detail the results of guideline studies with earthworms for the three commercial mixtures (European Chemical Bureau 2001; 2002; 2003). In short, no toxic effects on survival,

survival, reproduction or growth were seen at any the concentrations tested for any of the commercial mixtures.

The literature database detected two new studies with earthworms exposed to BDE-209. Another guideline study (OECD 222) also reported no effects on survival, reproduction or growth on earthworms exposed to up to 360 mg/kg dry soil (Xie et al. 2013a). The same research group had previously examined oxidative stress biomarkers in earthworms exposed to BDE-209 and found significant differences at concentrations above 0.1 mg/kg dry weight (Xie et al. 2011). In the absence of apical toxic effect, the ecological significant of such findings is uncertain.

5.2.3.2.4 Secondary poisoning

The toxicity to mammalian systems is reviewed in section 5.1.3 in the human perspective part of the review.

5.2.3.2.4.1 Birds

No toxicity data was available for birds in any of the European risk assessment reports (European Chemical Bureau 2001; 2002; 2003). A number of studies have since become available for BDE-209, commercial mixtures and other individual PBDE congeners and their results will be briefly presented here (Table 20).

The Annex XV dossier for BDE-209 mentions the only study available for BDE-209. Sifleet (2009) found that a median lethal dose of 44 µg when injected into chicken eggs during incubation. The experimental procedure is not a standard procedure and ECHA deemed that it did not replicate parental transfer. It is nonetheless the most common procedure applied in studies of the toxicity of PBDE commercial mixtures in birds. McKernan et al. (2009) examined the effects of the commercial mixture DE-71 on reproductive endpoints in three birds species; namely, the chicken, the mallard duck and the American kestrel. The chicken appears to be less sensitive to this pentaBDE mixture than BDE-209. No effects could be detected at the highest tested dose in the chicken and the mallard duck. Effects were seen however in the American kestrel at doses above 1 µg/g egg. This species appears to be particularly sensitive and it has been used extensively in many studies. Effects in that species have been seen on reproduction, growth and immunomodulation with commercial and synthetic PBDFE mixtures. Although an endocrine disrupting mechanism was suggested, attempts to detect effects on sex and thyroid hormones have remained elusive. The same applies to oxidative stress. Effects on retinol levels were however reported in one study with this species. The study by Fernie et al. (2009) used parental dietary exposure and was considered for the derivation of an EQS (Rodriguez-Romero 2011). This study validates concern over reproductive endpoints and suggests that courtship behaviour may be a particularly sensitive endpoint.

Effects on reproduction whilst failing to detect any effect on sex or thyroid hormones have also been seen in the European starling. There were however indications that hormone disruption or oxidative stress may play a role in common terns.

The only congener to have been studied in birds is BDE-99 (Eng et al. 2012; Eng et al. 2013; Murvoll et al. 2005; Winter et al. 2013). There was again evidence that reproduction is a sensitive endpoint, particularly reproductive behaviours, in the zebrafinch, but evidence for thyroid disruption or oxidative stress failed to materialise. A study with the domestic duck again pointed to a mechanism involving vitamin A and the retinol pathway.

Although on the basis of the current dataset, it would be difficult to comment on the potency of different PBDE congeners, it is not unreasonable to assume that they may affect similar endpoints by a similar mode of toxic action.

Table 20. Toxicity studies with BDE-209, pentaBDE commercial mixtures and BDE-99 in bird species

Test product	Exposure route	Species	Observed effects	Critical dose	Reference
BDE-209	Injection in the egg	Chicken	Egg mortality	20d-LD_{50} = 44 µg/egg (or 740 µg/kg ww)	Sifleet 2009
DE-71	Injection in the egg	Chicken	Embryonic survival, pipping and hatching success	NOEL > 20 µg/g egg	McKernan et al. 2009
		Mallard duck	Embryonic survival, pipping and hatching success	NOEL > 20 µg/g egg	McKernan et al. 2009
		American kestrel	Embryonic survival, pipping and hatching success	NOEL = 1 µg/g egg	McKernan et al. 2009
			Reproductive success and behaviour	NOEL = 3.01 ng/g (wet weight)	Marteinson et al. 2010; 2011
			Retinol levels in female and female nestlings	NOEL = 3 ng/g (wet weight)	Marteinson et al. 2010; 2011
	Parental dietary exposure	American kestrel	Eggshell thickness and reproductive success	75d-NOEL = 0.3 ppm	Fernie et al. 2009
			Courtship behaviour	75d-NOEL < 0.3 ppm	Fernie et al. 2008
PentaBDE	Silastic implants	European starling	Testosterone, estradiol and thyroid hormones	NOEL > 1740 µg/kg body weight	Van den Steen et al. 2010
			Higher egg weight and volume	NOEL < 150 µg/female	Van den Steen et al. 2009
Synthetic mixture	In ovo (injection) then dietary (nestlings)	American kestrel	Growth	<18.7 µg/egg then 15.6 ng/g bw/day	Fernie et al. 2006
			Immunomodulation	<18.7 µg/egg then 15.6 ng/g bw/day	Fernie et al. 2005a
			Thyroid, Vitamin A, Glutathione Homeostasis, and Oxidative Stress	> 1500 ng/g/egg then 15.6 ng/g/bird/day	Fernie et al. 2005b
		Common tern	Spleen weight, 8-Hydroxy-deoxyguanosine	NOEL = 0.2 µg/egg	Rattner et al. 2013
			Thyroid weight, humerus length, hepatic oxidative stress endpoints	NOEL < 20 µg/egg	Rattner et al. 2013

Table 20 cont.

Test product	Exposure route	Species	Observed effects	Critical dose	Reference
BDE-99	Injection in the egg	Zebrafinch	Clutch size in F1 birds (exposed in ovo)	NOEL = 2 ng/egg	Winter et al. 2013
		Domestic duck	Vitamin A status	Negative correlations but statistical significance not reported	Murvoll et al. 2005
	Oral dosing	Zebrafinch	Thyroid disruption, Oxidative stress	21d-NOEL > 173.8 ng/g body weight	Eng et al. 2013
			Male mating behaviour	21d-NOEL= 15.8 ng/g body weight	Eng et al. 2012

5.2.3.3 References

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5.2.4 Factors affecting mixture risk assessment for the environment

5.2.4.1 Empirical evidence on the mixture toxicity of PBDE-209 and lower-brominated PBDE-congeners

For the purposes of this report, the examination of existing mixture toxicity studies was confined to findings about the combined action of BDE-209 with other lower-brominated congeners. Mixtures of BDE-209 with other environmental chemicals were out of scope. The same applies to mixtures of lower-brominated PBDEs not including BDE-209.

As a result, the search identified just one single mammalian *in vitro* study, no evidence on combined effects in non-mammalian species, and no relevant studies on mixture effects in intact organisms.

Gregoraszczuk and co-workers (2008) studied effects on steroid secretion from porcine ovarian follicular cells. Cells were exposed to PBDE-47, 99, 100, and 209 individually and in combination. Concentration-response relationships were not determined but the substances were applied in just one single concentration each and in mixtures containing the same concentrations of the individual substances. Such an experimental design is inappropriate for quantitative comparisons of observed combined effects with predictions based on the non-interaction models of concentration addition or independent action (as defined in section 6.1 below). However, the design is sufficient to prove less-than-additive effects, if they should occur at the tested exposure levels and concentration ratios of mixture components.

The four tested PBDE congeners were all shown to cause increased testosterone and estradiol secretion from the cells, both individually and in combination. However, BDE-209 caused stronger effects when it was applied alone than in combination with the other three lower-brominated PBDEs. This suggests a less-than-additive activity in the *in vitro* assay used.

5.2.4.1.1 Consequences for mixture risk assessments

Rules laid down in Annex V to the POP Convention propose to assume dose (concentration) addition as a pragmatic default approach, unless there is documented evidence against additivity. No such evidence against additivity exists for hazardous effects in intact organisms. The documented evidence for less-than-additive effects of BDE-209 in combination with lower-brominated PBDEs in a single in *vitro study* is irrelevant for non-mammalian wildlife and it is considered to be insufficient for justifying the assumption of less-than-additive *in vivo* effects in mammalian wildlife. Such an assumption bears a strong risk for underestimating the actual risk and therefore requires a much higher weight of evidence.

5.2.4.2 References

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6. Mixture risk assessment

6.1 Introduction to mixture risk assessment

This section introduces the assessment concepts available to describe mixture toxicity and the mixture risk assessment (MRA) methods that are based on them. The two main assessment concepts are dose addition (DA) and independent action(IA), whilst the available MRA methods are almost exclusively based on the DA concept and include the hazard index (HI), point of departure index (PODI) and the toxic equivalency factor (TEF) approach (Kortenkamp et al. 2012; Kortenkamp, Backhaus, & Faust 2009).

6.1.1 Assessment concepts for mixture toxicity

Methods for experimental mixture studies can be divided into two major classes, “whole mixture approaches” and “component based” approaches. In whole mixture approaches, direct toxicological assessments of a given chemical mixture, such as a complex environmental sample are conducted by treating a mixture as if it were a single chemical. The composition of the mixture is not the topic of investigation, and whole mixture approaches do not require new, mixture-specific assessment concepts.

In component-based approaches, efforts are made to anticipate the effects of a mixture on the basis of the toxicity of its components. This makes it possible to draw more general conclusions about the relationship between the effects of single substances and those of their combinations. Numerous methods for this purpose have been described in the literature (see the overview in (EC 2009). Such methods allow quantitative predictions of mixture toxicities, without the need to test different mixture ratios, mixture concentrations or overwhelmingly large numbers of permutations of mixture components. They require information about the effects of the mixture components after administration as single chemicals and about their levels in the mixture (mixture ratio). The effects of all components must have been measured under the same conditions as the experimental mixture study, using the same toxicological endpoint. The experimentally observed mixture effects can then be compared with those expected on the basis of the effects of the components.

Among the component-based approaches, two fundamentally different concepts exist for the calculation of mixture effects on the basis of the toxicity of its components, independent action (IA) and dose or concentration addition (DA or CA). Both concepts rely on an additivity assumption, which is based on the expectation that all chemicals in the mixture exert their effects without influencing each other’s action. The additivity assumptions are not fulfilled when components of the mixture interact with one another, e.g. by undergoing chemical reactions with each other, or by inducing (de)toxifying metabolic conversions that target some or all of the mixture components.

The difference between IA and DA is in the way in which each concept constructs its additivity assumption. IA derives additivity assumptions from probabilistic considerations of the effects of the mixture components. In contrast, DA is based on the idea that all components in the mixture behave as if they are simple dilutions of one another, which is often taken to mean that DA describes the joint action of compounds with an identical mechanism of action.

6.1.1.1 Dose addition (DA)

DA (also known as concentration addition, CA) is based on the idea that all components in the mixture behave as if they are simple dilutions of one another, which is often taken to mean that DA describes the joint action of compounds with an identical mechanism of action. When chemicals interact with an identical, well-defined molecular target, it is thought that one chemical can be replaced totally or in part by an equal fraction of an equi-effective concentration (e.g. an EC50) of another, without changing

the overall combined effect. If the assumption of dose addition holds true, these fractions of equi-effective single substances concentrations – also called toxic units – simply sum up to an overall toxic unit of the mixture. Therefore, DA is also known as "Toxic Unit Summation". The concept can be mathematically formulated as:

$$ECx_{Mix} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1} \qquad \text{(Eq. 1)}$$

with n denoting the number of mixture components, p_i the relative fraction of chemical i in the mixture, and x a common effect level, provoked by an exposure to a single substance or mixture concentration ECx_{Mix} resp. ECx_i.

In general, no explicit formulation of the DA-expected mixture effect $E(c_{Mix})$ is possible. Direct calculations are restricted to the effect levels associated with the effect concentrations (*ECx*-values) (Faust et al. 2003).

The requirement of parallel dose response curves has often been used as a decision criterion about the application of DA to a specific mixture. However, the general formulation of DA in equation 1 does neither assume a specific shape of each concentration-response curve of the components, nor a specific relationship between the curves, such as parallelism. Even if all chemicals in a mixture share an identical receptor binding site, differences e.g. in the toxicokinetic behaviour of the substances might lead to concentration-response curves that are not parallel, yet DA may still apply.

6.1.1.1.1 Data requirements for using DA

To predict mixture effects by using DA, information about the doses that induce the same specific effect are required for both the mixture and all single components. For example, if the effect dose of a mixture leading to a 50% effect is known, then the equivalent effect doses (ED50) for all mixture components need to be available to reach decisions whether the combined effect is dose additive (see equation 1). In this case, the sum of toxic units in equation 1 will be 1. The same requirements need to be met for any other effect level. Usually, information about effect doses is accessible through dose-response analyses of the individual components in a mixture.

As with IA, NOAELs are strictly speaking not suited as input values for using DA, because NOAELs represent different (but unknown) effect doses. However, unlike IA, the measurement precision required for using the concept as the number of mixture components increases, does not change. This feature makes DA generally easier to use in most situations.

It is obvious from equation 1 that DA represents the weighted harmonic mean of the individual *ECx* values, with the weights being the fractions p_i of the components in the mixture. This has important favourable consequences for the statistical uncertainty of the DA-predicted joint toxicity. As the statistical uncertainty of the DA-predicted *ECx* for the mixture is the result of averaging the uncertainties of the single substance *ECx*-values, the stochastic uncertainty of the DA prediction is always smaller than the highest uncertainty found in all individual *ECx*-values. Perhaps contrary to intuition, the consideration of mixtures composed of a large number of agents actually reduces the overall stochastic uncertainty. This feature renders DA predictions quite reliable and robust.

6.1.1.1.2 Under DA, when is a mixture risk acceptable?

DA implies that every toxicant in the mixtures contributes in proportion to its toxic unit (i.e. its concentration and individual potency) to the mixture toxicity. Whether the individual doses are also effective on their own does not matter. Thus, combination effects should result from toxicants at or below effect thresholds, provided sufficiently large numbers of components sum up to a sufficiently high total dose. Unfortunately, that is often misunderstood to mean that mixture effects will arise with any combination of agents, if the principles of DA are fulfilled. However, this is not the case. For example,

the joint effect of two agents combined at 1/10 of their ADI is expected to be considerably smaller than the effect (if any) associated with the ADI of each of the chemicals on their own. Similarly, 100 chemicals combined at 1/100 of their ADI will not produce a mixture effect greater than the effects provoked at the ADI's of each of the single components (see equation 1).

6.1.1.2 Independent action (IA)

Independent action (sometimes also termed Effect Addition, Effect Multiplication or Abbotts Rule) conceptualises mixture effects by assuming that combined effects can be calculated from the effects caused by the individual mixture components on the basis of the statistical concept of independent random events (Bliss 1939). This can be mathematically expressed as:

$$E(c_{Mix}) = 1 - \prod_{i=1}^{n} [1 - E(c_i)] \qquad \text{(Eq. 2a)}$$

if the effect increases with increasing concentrations (e.g. when mortality data are considered) and

$$E(c_{mix}) = \prod_{i=1}^{n} E(c_i) \qquad \text{(Eq. 2b)}$$

when the effect decreases with increasing concentrations (when e.g. survival rates are observed). In both equations $E(c_{Mix})$ denotes the effect provoked by the total mixture at a concentration

$$c_{Mix} = \sum_{i=1}^{n} c_i \quad .$$

$E(c_i)$ are the effects that the individual components would cause if applied singly at that concentration at which they are present in the mixture.

Due to this probabilistic background, IA assumes strictly monotonic concentration-response curves of the individual mixture components and an Euclidian-type effect parameter scaled to an effect range of 0-1 (0-100%).

6.1.1.2.1 Applicability of IA to mixtures composed of agents with dissimilar modes of action

Theoretically, the stochastic principles of IA are also valid when one and the same agent is administered sequentially and irreversible events such as mortality are investigated. Because organisms cannot die twice, the probability expressed in equation 2 a,b applies, despite the fact that the mechanism by which the chemical provokes mortality is identical. In the case of simultaneous administration of many chemicals the principle of independent events can only be realised by making the additional assumption that all components in the mixture exert their effects by activating different effector chains that converge to produce a common effect. This is commonly thought to apply in cases where the chemicals in the mixture exert their effects through strictly independent, i.e. dissimilar mechanisms. By activating differing effector chains every component of a mixture of dissimilarly acting chemicals provokes effects independent of all other agents that might also be present, and this feature lends itself to statistical concepts of independent events.

6.1.1.2.2 Data requirements for using IA

IA uses single substance effects, $E(c_i)$, for predicting a mixture effect (equation 2 a, b). This means that the information need for utilising IA changes substantially with increasing numbers of mixture components. For example, according to IA a binary mixture of two agents that individually produce a 30% effect will lead to a 50% mixture effect. Thus, the application of IA means that effects of 30% have to be measured with reliability which usually does not present problems. In a 10-component mixture producing a 50% mixture effect, however, each component has to be present at a concentration that produces only a 6.7% individual effect. But effects of that magnitude are already at the borderline of what can be measured reliably in many *in vivo* toxicological experiments. The more compounds are present in a mixture, the lower the individual $E(c_i)$'s become that are required as input values for estimating a 50% mixture effect. The fact that increasingly lower $E(c_i)$-values for each component need to be measured for calculating IA-predictions is a serious drawback, as this increases experimental demands beyond what is technically achievable with the number of animals per does group normally used in toxicity studies.

NOAELs are not readily suited as input data for IA. NOAELs denote the highest tested doses that produced effects not statistically significantly different from those in untreated controls, but they do not describe effect magnitudes. Depending on the resolving power of the chosen experimental arrangement, the effects associated with NOAELs can be quite large, but cannot be measured directly, and are only accessible through regression modelling in dose-response analyses. However, the number of doses tested in studies that establish a NOAEL is often rather limited and does not permit regression analysis. As a result, it is normally not possible to establish whether a NOAEL is associated with a 5%, 10% or 20% effect. Consequently, the input data required for using IA are not accessible through reporting a NOAEL.

6.1.1.2.3 Under IA, when is a mixture risk acceptable?

Equations 2 a and b imply that agents present at doses associated with zero effects will not contribute to the joint effect of the mixture. If this condition is fulfilled for all components in the mixture no combination effect is expected under IA.

This means that claims of absence of mixture responses can only be substantiated if very small effects can be distinguished with reliability from zero effects. However, especially with mixtures composed of large numbers of components, this demands exceeds the resolving power of most toxicological studies which struggle to resolve 5% effects. According to IA, 10 components at doses associated with 5% effect will already produce a combination effect of 45%. Correspondingly, 100 agents with a 1% effect are expected to produce a mixture effect of 63%, and with 100 chemicals of 0.1% effect the expected joint response under IA will still be 9.5 %. Such small effects can only be demonstrated with astronomically large numbers of animals.

6.1.1.2.4 Empirical evidence for IA

The use of DA as a default assumption would be challenged if there were empirical evidence that IA would be a suitable alternative. Systematic literature searches were carried out to identify examples when IA provided an accurate prediction of a mixture effect, in a situation in which the predictions under DA and IA were separable; when IA provided a prediction that was more conservative than the DA prediction; and when IA provided a more conservative prediction AND was also accurate.

The ecotoxicology literature contains a few examples when the effects of carefully designed mixtures were shown to validate the IA model (Kortenkamp, Backhaus, & Faust 2009). Of importance is a study of 16 biocides whose combination effect on algal toxicity was accurately predicted by IA (Faust et al. 2003). These components were selected on the basis of their strictly different specific mechanism of action. Faust et al. also found that whilst IA was the accurate model, in this case the predicted effect under DA was greater than that under IA, and so DA could be considered the conservative model. If this finding is generally applicable then there would be reassurance that even in cases when IA is valid, DA

can still provide a conservative risk estimate, supporting the use of DA as the default concept in mixture risk assessment.

A proof-of-principle example of the validity of IA has not been identified in the mammalian toxicology literature (Kortenkamp et al. 2012; Kortenkamp, Backhaus, & Faust 2009). The reason for this is likely to be the difficulties, including costs and ethical considerations, of performing mammalian studies with a sufficiently large number of components (which is required to allow the predictions of IA and DA to be distinguished from each other) and the difficulty in selecting an appropriate effect for which there are enough well characterised chemicals with strictly different specific mechanisms of action. The amount and level of knowledge required to design a mixture experiment that is suitable to test the hypothesis that IA is accurate in a mammalian system appears to be far greater than the knowledge that is typically available for chemicals.

A situation when IA was both more conservative than DA, and also accurate was not identified in the literature. The factors that determine whether IA produces a more conservative prediction than DA include the number of mixture components, the slope of the individual dose response curves; the mixture ratio of components in the mixture and the effect level under consideration.

6.1.1.3 Toxicological interactions

Toxicological interactions have been defined as "any toxic responses that are greater than or less than what is observed under an assumption of additivity." (EPA 2000). In this context, 'additivity' is typically the most appropriate concept from a choice of DA or IA. Interactions that results in an effect less than expected under additivity are referred to as antagonism, sub-additivity or inhibition, and those resulting in an effect greater than predicted are described as synergy or supra-additivity.

Consideration of interactions is important because the use of component-based approaches, such as DA or IA, assumes the absence of interactions. A very relevant question is how much the occurrence of an interaction could alter the mixture effect from that predicted under additivity. Of most concern is a possible synergy, or supra-additive interaction, that would increase the level of toxicity compared to that expected. The literature relating to this question has been critically reviewed (Boobis et al. 2011). Boobis *et al.* reviewed the experimental evidence for synergies at low doses (defined as doses close to points of departure for individual chemicals) in mixture studies and identified 90 studies, of which only 11 studies reported a quantitative estimate of a low-dose synergy. Three criteria were identified to make the quantification of synergy more consistent: synergy should be defined as departure from the mixture prediction using DA; a uniform procedure should be developed/used to assess synergy at low doses; and the method used to define the POD used to assess synergy should be standardised.

Only 6 studies were considered to provide a useful quantitative estimate of synergy and these comprised three studies of binary mixtures, two studies of five component mixtures and one study of an 18 component mixture. When the magnitude of synergy was calculated based on the ratio of observed to predicted dose for a fixed response ("Method A") or the ratio of observed to predicted response for a fixed dose ("Method B") it was found that the magnitude of synergy at low doses did not exceed that of the prediction made using DA by greater than a factor of 4. The number of studies identified was not great enough to allow comparison of the effect of using method A or B on the observed synergy.

Boobis *et al.* noted that the role of interactions is the subject of continued debate amongst scientists and risk assessors, and that there is incomplete agreement on the impact of interactions following exposure to a chemical mixture. Given the results of their review, Boobis *et al.* considered that "*there is probably merit in the default regulatory approaches that assume toxicological interactions are not likely to occur at the low dose permitted under existing exposure standards*" however they acknowledged that this could not be a firm conclusion, especially for the effects of cumulative and low-level chronic exposure, and that, although the magnitude of observed synergies appears to be low, more work is required to determine the frequency of synergy in real world situations (Boobis et al. 2011).

The presence of significant, unpredictable synergies could have questioned the use of any additivity concept, including DA, as a default in mixture risk assessment. However if significant synergies can be considered unlikely, as is indicated, then the suitability of DA as a conservative default is unaffected.

6.1.2 Mixture risk assessment (MRA) methods

The evaluation of experimental data describing the combined effects of chemicals, referred to as ***mixture effect assessment***, has to be distinguished clearly from approaches employed for conducting mixture risk assessment in practice, referred to in this report as ***mixture risk assessment (MRA) methods***.

The application of MRA methods requires clarity about the goal of the assessment. The aim can be to arrive at a risk estimate, an estimation of safe levels, of margins of exposure, or can consist of ways of prioritizing certain mixtures, for further study or for regulatory interventions. Estimations of safe levels or margins of exposure may be based on worst-case-assumptions, but the prioritization of mixtures (or affected sites) has to rely on fairly accurate quantitations of risk.

Almost all MRA methods in current use are applications of the concept of dose addition. These include the Hazard Index (HI), Toxic Unit Summation (TUS), Point of Departure Index (PODI), Relative Potency Factors and the TEQ concept.

Methods explicitly derived from independent action are not developed. An implicit application of independent action is the assumption that mixture effects will not arise when all chemicals in question are present at levels below their ADIs, with the additional implicit assumption that ADIs represent true zero effect levels. It should be emphasised that the implicit application of independent action can only be used for chemicals for which ADIs have been derived. However, this is only the case for a small minority of chemicals in current use.

6.1.2.1 Approaches based on dose addition (DA)

6.1.2.1.1 Hazard Index

The Hazard Index (HI) (Teuschler & Hertzberg 1995) is a regulatory approach to component-based mixture risk assessment derived from DA and which can be generally defined by the formula

$$HI = \sum_{i=1}^{n} \frac{EL_i}{AL_i}$$

where *EL* is the exposure level, AL is the acceptable level, and *n* is the number of chemicals in the mixture. Various measures for exposure levels and expectable levels may be applied; the only constraint is that EL and AL must be expressed in the same unit. Input values for AL can be ADIs or reference doses (RfD) for specific endpoints.

If HI > 1, the total concentration (or dose) of mixture components exceeds the level considered to be acceptable. The method offers flexibility in applying different UFs when defining AL for the individual substances.

An assumption implicit in the use of the HI approach, and one that derives from the principles of the DA concept, is that the acceptable levels AL for each individual chemical represent exposures associated with the same (small or negligible) effect. In most cases, this is not proven in practice, and will remain

unproven in the foreseeable future. For most practical applications, however, the error in making this assumption can be considered small.

6.1.2.1.2 Toxic Unit Summation

The method of Toxic Unit Summation (TUS) (Sprague 1970) is a direct application of the DA concept and defined by the formula

$$TUS = \sum_{i=1}^{n} TU_i = \sum_{i=1}^{n} \frac{c_i}{ECx_i}$$

where c_i are the actual concentrations (or doses) of the individual substances in a mixture and ECx_i denote equi-effective concentrations (or doses) of these substances if present singly (e.g. $EC50_i$). The quotients c_i / ECx_i are termed Toxic Units (TU). Toxic Units rescale absolute concentrations (or doses) of substances to their different individual toxic potencies. They express the concentrations (or doses) of mixture components as fractions of equi-effective individual concentrations (or doses) ECx_i. Typically, x = 50 % ($EC50_i$) is chosen as the reference level, but TUS can also be calculated for any other effect level x. If TUS = 1, the mixture is expected to elicit the total effect x. If the sum of Toxic Units is smaller or larger than 1, the mixture is expected to elicit effects smaller or larger than x, respectively.

6.1.2.1.3 Point of Departure Index

The Point of Departure Index (PODI) is an approach to component-based mixture risk assessment which is similar to the HI and TUS. In contrast to the HI, however, exposure levels (EL) of chemicals in a mixture are not expressed as fractions of individually acceptable levels (AL) but as fractions of their respective points of departure (PODs) such as NOAELs or benchmark concentrations or doses (BMD). In this way, different uncertainty factors that may be included in AL values (see HI) are removed from the calculation (Wilkinson et al. 2000):

$$PODI = \sum_{i=1}^{n} \frac{EL_i}{POD_i}$$

A PODI lends itself to the estimation of margins of exposure for the mixture of interest. Similar to the HI, there is the implicit assumption that all PODs are associated with the same effect magnitude, a principle derived from the features of DA.

6.1.2.1.4 Relative Potency Factors

The Relative Potency Factor (RPF) approach is another application of the DA concept for mixtures of chemical substances that are assumed to be toxicologically similar (EPA 2000). The concentrations (or doses) of mixture components are scaled relatively to the concentration (or dose) of an index compound, and then summed up. The scaling factor is called RPF. The total toxicity of the mixture is assessed in terms of the toxicity of an equivalent concentration of the index compound. In general, the mixture concentration C_m expressed in terms of the index compound for n compounds is

$$C_m = \sum_{i=1}^{n} (c_i * RPF_i)$$

where c_i is the concentration of the i^{th} mixture component, and RPF_1 = 1, as i = 1 indicates the index chemical.

6.1.2.1.5 Toxic Equivalency Factors

The Toxic Equivalence Factor (TEF) is a specific type of RPF formed through a scientific consensus procedure (EPA 2000). Based on the assumptions of a similar mechanism of action of structurally related chemicals and parallel concentration (or dose) response curves, they were first developed for dioxins. The total toxicity of the mixture is assessed in terms of the toxicity of an equivalent concentration of an index compound. The total equivalent quantity *TEQ* is estimated by summation of the concentrations (or doses) of mixture components c_i multiplied by the respective TEF_i:

$$TEQ = \sum_{i=1}^{n} (c_i * TEF_i)$$

6.1.2.2 Data requirements and applicability of the mixture risk assessment methods

All of the above mixture risk assessment methods require at least rudimentary dose-response information of individual mixture components which is used to derive the input values, be they ADIs, RfDs, POD or information about relative potencies such as RPF or TEF. Information about exposures must also be available.

The HI sums up ratios of exposure levels and ADIs or RfDs over chemicals. These estimates can be arrived at by utilizing different uncertainty factors (UF) for each mixture component, in order to deal with differences in data quality and sources of uncertainty.

If this is perceived to be inadequate, the PODI method can be used. PODI is based not on reference doses, but on points of departure (NOAELs, benchmark doses). Extrapolation issues (e.g. animal to human) are dealt with either by using one overall UF, or by estimating margins of exposure.

The TEQ concept is predicated on the choice of a reference chemical and requires parallel dose-response curves for all components. Both these requirements are often not met by chemicals, but the method has been validated for dioxins and dioxin-like substances.

6.1.2.3 Approaches based on independent action (IA)

In general, MRA approaches based on IA are much less available than approaches based on DA, see above. Approaches such as the Hazard Index or Toxic Equivalency Factors do not have counterparts founded on IA principles and, because of the difference in formulation of DA and IA, similar approaches may not be conceivable.

One pragmatic application of IA is the stance that a mixture effect will not occur if each component is present at or below its individual zero effect level. However, this rests on the use of true zero effect levels, whose identification may be controversial, and should not be applied when effects are present but cannot be measured (when they are below the statistical detection limits of the assay). This issue has been thoroughly reviewed elsewhere (EC 2009). Simplified implementations of IA that are sometimes mooted are the notions that 1) the mixture effect is equal to the effect of the most potent component or that 2) the mixture effect is equal to the summation of the effects of the components. These approaches appear to rely on assumptions about the correlation of susceptibility to the mixture components, these assumptions being rarely stated and potentially hard to substantiate (Kortenkamp et al. 2012).

There would appear to be no practical approach for the use of IA in MRA, other than the assumption that mixture effects will not occur if the individual components are without effect.

6.1.3 References

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6.2 Mixture risk assessment for human / mammalian toxicity of PBDEs

6.2.1 Purpose of assessment

In this section, we conduct a MRA for combinations of PBDEs identified as being relevant for human exposures. We will utilize a tiered assessment, using the WHO / IPCS framework developed and described by Meek et al. (2011).

The analysis will proceed in a step-wise fashion, as follows:

- First, it is necessary to resolve which PBDE should be grouped together and subjected to MRA, and which criteria should be used to achieve these groupings.
- Second, a suitable MRA method will have to be selected, in line with the features of available input data.
- Finally, the MRA will be conducted, and the outcome interpreted.

6.2.2 Grouping of PBDE into a common assessment group for MRA

6.2.2.1 Common adverse outcomes

Before MRA can be conducted, it is necessary to evaluate whether the chemicals of interest produce a common adverse outcome. As established in the section on human toxicology (5.1.3), many PBDE congeners induce neurodevelopmental toxicity in rodents. For many PBDE this is also the critical toxic endpoints that can form the basis for deriving reference doses (EFSA 2011).

Although the mechanisms that underlie this kind of toxicity remain to be fully established, the available evidence points to disruption of thyroid hormones and direct toxicity to neuronal cells as the mechanisms involved in developmental neurotoxicity. Although direct evidence that several PBDE can act together to affect this critical endpoint is missing, it is plausible to assume that they will exhibit joint toxicity through these mechanisms.

6.2.2.2 Common assessment groups

On the basis of experimental data on their ability to induce neurodevelopmental effects in rodents, the following PBDE congeners can be grouped together to be subjected to MRA:

BDE-47, -99, -153, -183, -203, -206, -209.

Toxicity data supporting the assignment of other PBDE to this grouping are missing, and this data gap will have to be bridged during the application of a MRA method.

6.2.3 Selecting a MRA method

Considering the mechanisms debated as relevant for the induction of developmental neurotoxicity it is reasonable to assume that dose addition will be the appropriate assessment concept for the approximation of combined neurodevelopmental effects of PBDE. Accordingly, the Hazard Index (HI) approach and the Point of Departure Index (PODI) suggest themselves as suitable MRA methods. The conceptually related Toxic Unit Summation (TUS) method is not normally used in connection with reference doses derived for human risk assessment and is better suited for the evaluation of

experimental data. The remaining other two applications of dose addition, the Relative Potency Factor (RPF) method and the Toxicity Equivalency (TEQ) method can only be used for substances that exhibit parallel dose-response curves. Since this requirement is not fulfilled with PBDEs, these two methods will not be considered further.

Thus, the choice is between the HI and the PODI method.

As described in section 5.1.3.3.5, EFSA (2011) defined critical human intake values that were based on points of departures (POD), here $BMDL_{10}$, estimated for neurodevelopmental toxicity in animal studies. EFSA used these values to establish Margins of Exposure (MoE) for each BDE congener in isolation. In principle, combinations of several BDEs could be assessed by using these values as input for the PODI method, with the aim of assessing an overall MoE for the combination. This method would be practicable, if the same MoE could be applied for all BDE congeners of interest. However, this is not the case, as follows:

For BDE-47, -99 and -153, EFSA used the rodent $BMDL_{10}$ for deriving acceptable exposure levels by toxicokinetic modelling with body burden as the dose metric (see 5.1.3.3.5) and considered a MoE of 2.5 as adequate. However, in the case of BDE-209, the CONTAM Panel did not use body burden calculations, and instead compared the BDE-209 $BMDL_{10}$ directly with human intake values. For this approach a MoE of 100 or larger is normally considered adequate. However, this means that the POD used by EFSA cannot be aggregated by using the PODI method.

The only option that is left is to use EFSAs congener specific critical human intake values to define reference doses by applying uncertainty factors that reflect the respective minimal MoE. In line with the evaluation approach taken by EFSA, an uncertainty factor of 2.5 will be used for BDE-47, -99 and -153, and for BDE-209, a factor of 100 is required. It should be stressed that these uncertainty factors are not the most conservative. In view of the sensitive life stages involved (young children) it can be argued that larger uncertainty factors are required for all congeners. However, for the purposes of this MRA, values similar to the EFSA MoE evaluation will be used. Table 21 details the conversions used to translate the EFSA values into reference doses useful as inputs for the HI method.

Table 21: Reference doses for PBDE congeners

Congener	Critical intake derived by EFSA (2011) (ng/kg d)	Uncertainty factor (equivalent to minimal MoE)	Tolerable intake (ng/kg d)
BDE-47	172	2.5	68.8
BDE-99	4.2	2.5	1.68
BDE-153	9.6	2.5	3.84
BDE-209	1,700,000	100	17000

6.2.4 Input values for MRA

Input values for the chosen HI method are:

- Congener-specific estimated human intakes for all relevant exposure media and routes of exposure
- Congener-specific reference doses describing “tolerable” human intakes, either derived from the body burden method, or using traditional dose metrics, as listed in Table 21.

The congener-specific human intake estimates represent upper bounds, in line with the practice in EFSA (2011). These are upper bounds of means of compilations, and not worst-case assumptions.

Table 22: Estimated total PBDE intakes for different age groups (ng/kg d)

Upper bounds

Age class	Route	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-196	BDE-197	BDE-201	BDE-202	BDE-203	BDE-206	BDE-207	BDE-208	BDE-209	Total	Fraction
Infants	**Breast feeding, average milk consumption**	**0.83**	**16**	**5**	**3**	**8.65**	**1.46**	**1.38**	**0.55**	**2.16**	**0.56**	**0.46**	**0.55**	**2.53**	**3.95**	**1.15**	**13.3**	**61.5**	1
< 1 year	**Breast feeding, high milk consumption**	**1.25**	**24**	**7.5**	**4.5**	**13**	**2.2**	**2.1**	**0.83**	**3.24**	**0.84**	**0.69**	**0.83**	**3.8**	**5.93**	**1.73**	**19.95**	**92.4**	1
Children 0.5-3	Food (EFSA 2011)	0.87	6.4	2.99	1.86	1.62	1.81	1.56									9.69	26.8	0.23
years	Dust	0.49	9.29	12.20	2.60	1.84	1.53	1.36	0.39	0.43				2.37	1.67	0.83	56.04	91.0	0.77
	Total	**1.36**	**15.69**	**15.19**	**4.46**	**3.46**	**3.34**	**2.92**	**0.39**	**0.43**				**2.37**	**1.67**	**0.83**	**65.73**	**117.8**	
Adults	Food (EFSA 2011)	0.28	1.91	0.65	0.7	0.42	0.51	0.36									2.82	7.7	0.44
	Dust	0.05	1.00	1.31	0.28	0.20	0.16	0.15	0.04	0.05				0.25	0.18	0.09	6.01	9.8	0.56
	Total	**0.33**	**2.91**	**1.96**	**0.98**	**0.62**	**0.67**	**0.51**	**0.04**	**0.05**				**0.25**	**0.18**	**0.09**	**8.83**	**17.4**	
Adults	Food (EFSA 2011)	0.28	1.91	0.65	0.7	0.42	0.51	0.36									2.82	7.7	0.26
	additional fish intake	0.23	5.36	0.75	2.07	0.47	0.59	0.58						0.08	0.15	0.05	1.77	12.1	0.41
	Dust	0.05	1.00	1.31	0.28	0.20	0.16	0.15	0.04	0.05				0.25	0.18	0.09	6.01	9.8	0.33
	Total	**0.56**	**8.27**	**2.71**	**3.05**	**1.09**	**1.26**	**1.09**	**0.04**	**0.05**				**0.34**	**0.33**	**0.14**	**10.60**	**29.5**	
Adults total Lorber 2008		0.029	1.971	2.186	0.829	0.23	0.150	0.064									2.114		

Table 23: Tier 1 MRA for PBDE

use reference dose for the most toxic congener, BDE-99 **Reference dose (BDE-99) = 1.68 ng/kg d**

use upper bound intake estimates for each age group

apply

HI

Tier 1: Infants <1 year	**BDE-28**	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**	**BDE-183**	**BDE-196**	**BDE-197**	**BDE-201**	**BDE-202**	**BDE-203**	**BDE-206**	**BDE-207**	**BDE-208**	**BDE-209**
Intake (ng/kg d)	1.25	24	7.5	4.5	13	2.2	2.1	0.83	3.24	0.84	0.69	0.83	3.8	5.93	1.73	19.95
Reference dose BDE-99: 1.68 ng/kg d	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68
Hazard Quotient HQ	0.74	14.29	4.46	2.68	7.74	1.31	1.25	0.49	1.93	0.50	0.41	0.49	2.26	3.53	1.03	11.88
HI = sum of HQ	**55**															

Tier 1: Children 0.5 - 3 years	**BDE-28**	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**	**BDE-183**	**BDE-196**	**BDE-197**	**BDE-201**	**BDE-202**	**BDE-203**	**BDE-206**	**BDE-207**	**BDE-208**	**BDE-209**
Intake (ng/kg d)	1.36	15.69	15.19	4.46	3.46	3.34	2.92	0.39	0.43				2.37	1.67	0.83	65.73
Reference dose BDE-99: 1.68 ng/kg d	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68
Hazard Quotient HQ	0.81	9.34	9.04	2.65	2.06	1.99	1.74	0.23	0.25	0.00	0.00	0.00	1.41	0.99	0.49	39.12
HI = sum of HQ	**70**															

Tier 1: Adults with additional high fish intake	**BDE-28**	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**	**BDE-183**	**BDE-196**	**BDE-197**	**BDE-201**	**BDE-202**	**BDE-203**	**BDE-206**	**BDE-207**	**BDE-208**	**BDE-209**
Intake (ng/kg d)	0.56	8.27	2.71	3.05	1.09	1.26	1.09	0.04	0.05				0.34	0.33	0.14	10.60
Reference dose BDE-99: 1.68 ng/kg d	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68
Hazard Quotient HQ	0.34	4.92	1.61	1.81	0.65	0.75	0.65	0.02	0.03	0.00	0.00	0.00	0.20	0.20	0.08	6.31
HI = sum of HQ	**17.5**															

Table 24: Tier 2 MRA for PBDE

use upper bound intake estimates for each age group
use reference doses for BDE-47, -99, -153 and -209; apply BDE-47 value to BDE-28, apply BDE-99 value to BDE-100, apply BDE-153 value to BDE-154, apply BDE-209 value to all congeners from BDE-183 to -208
apply HI

Tier 2: Infants <1 year	**BDE-28**	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**	**BDE-183**	**BDE-196**	**BDE-197**	**BDE-201**	**BDE-202**	**BDE-203**	**BDE-206**	**BDE-207**	**BDE-208**	**BDE-209**
Intake (ng/kg d)	1.25	24	7.5	4.5	13	2.2	2.1	0.83	3.24	0.84	0.69	0.83	3.8	5.93	1.73	19.95
Reference dose (ng/kg d)	68.8	68.8	1.68	1.68	3.84	3.84	17000	17000	17000	17000	17000	17000	17000	17000	17000	17000
Quotient	0.02	0.35	4.46	2.68	3.39	0.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HI = sum of HQ	**11.5**															

Tier 2: Children 0.5 - 3 years	**BDE-28**	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**	**BDE-183**	**BDE-196**	**BDE-197**	**BDE-201**	**BDE-202**	**BDE-203**	**BDE-206**	**BDE-207**	**BDE-208**	**BDE-209**
Intake (ng/kg d)	1.36	15.69	15.19	4.46	3.46	3.34	2.92	0.39	0.43				2.37	1.67	0.83	65.73
Reference dose (ng/kg d)	68.8	68.8	1.68	1.68	3.84	3.84	17000	17000	17000	17000	17000	17000	17000	17000	17000	17000
Quotient	0.02	0.23	9.04	2.65	0.90	0.87	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HI = sum of HQ	**14**															

Tier 2: Adults with additional high fish intake	**BDE-28**	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**	**BDE-183**	**BDE-196**	**BDE-197**	**BDE-201**	**BDE-202**	**BDE-203**	**BDE-206**	**BDE-207**	**BDE-208**	**BDE-209**
Intake (ng/kg d)	0.56	8.27	2.71	3.05	1.09	1.26	1.09	0.04	0.05				0.34	0.33	0.14	10.60
Reference dose (ng/kg d)	68.8	68.8	1.68	1.68	3.84	3.84	17000	17000	17000	17000	17000	17000	17000	17000	17000	17000
Quotient	0.01	0.12	1.61	1.81	0.28	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HI = sum of HQ	**4.2**															

Table 25: Tier 3 MRA for PBDE

use reference doses for BDE-47, -99, 153, -209, leave BDE-28 and -100 unassigned, apply BDE-153 value to BDE-154,
apply BDE-209 value to BDE-183, -196, -197, -201, -202, -203, -207, 208
use highest available intake estimates for each age group
apply HI

Tier 3: Infants <1 year	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-196	BDE-197	BDE-201	BDE-202	BDE-203	BDE-206	BDE-207	BDE-208	BDE-209
Intake (ng/kg d)	1.25	24	7.5	4.5	13	2.2	2.1	0.83	3.24	0.84	0.69	0.83	3.8	5.93	1.73	19.95
Reference dose (ng/kg d)		68.8	1.68		3.84	3.84	17000	17000	17000	17000	17000	17000	17000	17000	17000	17000
Quotient		0.35	4.46		3.39	0.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HI = sum of HQ	**8.8**															

Tier 3: Children 0.5 - 3 years	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-196	BDE-197	BDE-201	BDE-202	BDE-203	BDE-206	BDE-207	BDE-208	BDE-209
Intake (ng/kg d)	1.36	15.69	15.19	4.46	3.46	3.34	2.92	0.39	0.43				2.37	1.67	0.83	65.73
Reference dose (ng/kg d)		68.8	1.68		3.84	3.84	17000	17000	17000	17000	17000	17000	17000	17000	17000	17000
Quotient		0.23	9.04		0.90	0.87	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HI = sum of HQ	**11**															

Tier 3: Adults with additional high fish intake	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-196	BDE-197	BDE-201	BDE-202	BDE-203	BDE-206	BDE-207	BDE-208	BDE-209
Intake (ng/kg d)	0.56	8.27	2.71	3.05	1.09	1.26	1.09	0.04	0.05				0.34	0.33	0.14	10.60
Reference dose (ng/kg d)		68.8	1.68		3.84	3.84	17000	17000	17000	17000	17000	17000	17000	17000	17000	17000
Quotient		0.12	1.61		0.28	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HI = sum of HQ	**2.4**															

Table 26: Tier 4 MRA for PBDE

use reference doses for BDE-47, -99, 153, -209, leave BDE-28, -100 and -154 unassigned,
apply BDE-209 value to BDE-183, -196, -197, -201, -202, -203, -207, 208
use only intake via food, EFSA (2011) estimates
apply HI

Tier 4: Infants <1 year, average breast milk consumption	**BDE-28**	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**	**BDE-183**	**BDE-196**	**BDE-197**	**BDE-201**	**BDE-202**	**BDE-203**	**BDE-206**	**BDE-207**	**BDE-208**	**BDE-209**
Intake from breastfeeding (ng/kg d)	0.83	16	5	3	8.65	1.46	1.38	0.55	2.16	0.56	0.46	0.55	2.53	3.95	1.15	13.3
Reference dose (ng/kg d)		68.8	1.68		3.84		17000	17000	17000	17000	17000	17000	17000	17000	17000	17000
Quotient		0.23	2.98		2.25		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HI = sum of HQ	**5.46**															

Tier 4: Children 0.5 - 3 years, exposure via food only	**BDE-28**	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**	**BDE-183**	**BDE-196**	**BDE-197**	**BDE-201**	**BDE-202**	**BDE-203**	**BDE-206**	**BDE-207**	**BDE-208**	**BDE-209**
Intake from food (ng/kg d)	0.87	6.40	2.99	1.86	1.62	1.81	1.56									9.69
Reference dose (ng/kg d)		68.8	1.68		3.84		17000	17000	17000	17000	17000	17000	17000	17000	17000	17000
Quotient		0.09	1.78		0.42		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HI = sum of HQ	**2.3**															

Tier 4: Adults, no additional fish consumption	**BDE-28**	**BDE-47**	**BDE-99**	**BDE-100**	**BDE-153**	**BDE-154**	**BDE-183**	**BDE-196**	**BDE-197**	**BDE-201**	**BDE-202**	**BDE-203**	**BDE-206**	**BDE-207**	**BDE-208**	**BDE-209**
Intake, no additional fish consumption (ng/kg d)	0.33	2.91	1.96	0.98	0.62	0.67	0.51	0.04	0.05				0.25	0.18	0.09	8.83
Reference dose (ng/kg d)		68.8	1.68		3.84		17000	17000	17000	17000	17000	17000	17000	17000	17000	17000
Quotient		0.04	1.17		0.16		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HI = sum of HQ	**1.37**															

Table 26: Tier 4 MRA for PBDE

Tier 4: Adults, intake via food only, no additional fish consumption	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-196	BDE-197	BDE-201	BDE-202	BDE-203	BDE-206	BDE-207	BDE-208	BDE-209
Intake via food (ng/kg d)	0.28	1.91	0.65	0.70	0.42	0.51	0.36									2.82
Reference dose (ng/kg d)		68.8	1.68		3.84		17000	17000	17000	17000	17000	17000	17000	17000	17000	17000
Quotient		0.03	0.39		0.11		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
HI = sum of HQ	**0.52**															

6.2.5 Applying a tiered assessment framework

We adapted the tiered framework analysis developed by WHO/IPCS (Meek et al. 2011) to suit a MRA for PBDE combinations. This analysis takes a step-wise approach by initially utilising quite crude but conservative assumptions at the initial tiers. If these crude assumptions do not result in indications of risks (i.e. the HI does not exceed 1) the analysis is discontinued. If however the HI is larger than 1, the analysis must proceed by continuously refining the underlying assumptions.

However, in the case of PBDEs, the assumptions concerning congener-specific intakes are already quite refined. In contrast, only few congeners have been evaluated toxicologically to a sufficient standard. For this reason, the simplifying assumption made in the following tired assessment are mostly concerned with bridging data gaps about tolerable human intakes for PBDE congeners.

6.2.6 Results

The PBDE intake values used for all age groups in connection with the HI method are shown in Table 22. These are the same values as shown in Table 6 in section 5.1.2.3. In line with the conservatism required for MRA these are upper bound mean values from several sources, but not worst-case assumptions. Accordingly, the adult intake estimates were based on people with high fish consumption. These intake estimates will form the basis for the MRA in all tiers, except the last tier, Tier 4.

6.2.6.1 Tier 1 analysis

The Tier 1 MRA analysis is shown in Table 23. It includes all PBDE congeners for which reasonable intake estimates were made. However, for many of the congeners in this intake assessment, reference doses describing «tolerable» intakes are not available. Without such reference doses, a mixture risk assessment according to the HI method is not possible. One solution would be to leave out congeners for which such reference doses are not available. But by implication this would equate to the assumption that these congeners are not toxic. For a Tier 1 assessment however, such an assumption is insufficiently conservative. The alternative is to initially assume that all congeners are as toxic as the most potent of all congeners, BDE-99, which was the assumption followed here. Accordingly, the BDE-99 reference dose of 1.68 ng/kg d (see Table 21) was applied uniformly to all BDE congeners. As shown in Table 23, this produced quite high HIs of 55, 70 and 17.5 for infants, young children and adults with high fish consumption, respectively.

Since these His exceed 1 by a large margin, a refinement of the analysis is called for. This can be achieved by relaxing the assumption that all congeners are as potent as the most potent BDE-99, and by adopting the reference doses depicted in Table 21.

6.2.6.2 Tier 2 analysis

Accordingly, Tier 2 utilised the congener-specific reference doses for BDE-47, -99, -153 and -209 listed in Table 21. However, this left BDE-28, -100, -154, -183 and all other higher brominated congeners (apart from BDE-209) without a reference dose. Leaving these congeners without a reference dose is equivalent to the assumption that they are without effect. Since this was deemed insufficiently conservative for a Tier 2 analysis, BDE-28 was assumed to be as potent as BDE-47 and accordingly the reference dose for BDE-47 was used also for BDE-28. Similarly, BDE-100 was assumed to be as toxic as -99 and the reference dose for BDE-153 was used also for -154. The remaining higher brominated congeners were assumed to be as toxic as BDE-209, and the BDE-209 tolerable intake was used for all these congeners.

As was to be expected, the use of these reference doses led to somewhat smaller HI (Table 24). However, the HI derived for all age groups were still substantially larger than 1. This provided the stimulus for further relaxation of the conservative assumptions adopted thus far. An observation from Table 24 is that the higher brominated congeners including BDE-209 which were assigned the reference dose for BDE-209 do practically not contribute to the HI.

6.2.6.3 Tier 3 analysis

In Tier 3 of the MRA the toxicologically not well assessed BDE-28 and -100 were left without a reference dose, which is equivalent to assuming that these congeners do not contribute to a joint effect. BDE-154 was assumed to be of equal potency as BDE-153.

As can be seen in Table 25, these changes decreased the HI somewhat, compared to Tier 2 analysis, but the resulting HI were still substantially larger than 1. For infants, the HI was 8.8, for young children 11 and for adults engaging in high fish consumption an HI of 2.4 was calculated.

Although the scope for further refinements is limited, we conducted a Tier 4 analysis in which different assumptions about PBDE exposures were introduced.

6.2.6.4 Tier 4 analysis

In Tier 4, only BDE congeners with a reference dose were used, i.e. BDE-47, -99, -153 and -209. The congeners from BDE-183 to -208 were evaluated using the reference dose for BDE-209, as before (Table 26).

The only option left for refinement was to introduce altered intake estimates.

Accordingly, we assumed that breastfed infants have average, and not high breast milk consumption. This reduced the HI to 5.4, still far higher than 1.

For children of age 0.5 – 3 years, we assumed exposure via food only, and neglected the intake via dust. This rather unrealistic assumption reduced the HI to 2.3, also higher than 1.

Similarly, the disregard for additional PBDE exposures via fish for adults lowered the HI to 1.37. Only when the adult exposure via additional fish consumption and dust was ignored, and only exposure via food taken into account, was the HI decreased to a value of 0.52, not signalling risk.

For all age groups, the dominant contribution to the HI came from BDE-99, and for infants and young children additionally from BDE-153.

6.2.7 Conclusions

The MRA for combined exposures to several PBDEs in humans shows that acceptable levels are exceeded for all age groups, particularly for small children. Considering that the analysis was not based on very conservative reference values for congener-specific tolerable intakes, this might warrant health concerns. With the traditional congener-by-congener risk assessment such concerns would remain undetected, except for single exposures to BDE-99 and -153 for infants and BDE-99 for young children and adults engaging in high fish consumption.

The MRA for combined human PBDE intakes has driven risk estimates upwards, compared with single-congener assessments. It could be argued that the limitations of this analysis lie in the absence of empirical evidence *in vivo* of combined effects of several PBDEs. However, *in vitro* studies with neuroblastoma cells have given indications of synergistic effects with BDE-47 and -99, especially in the low dose range. Furthermore, it is plausible to assume that BDEs will work together to produce combination effects, considering the common adverse developmental neurotoxicity outcomes induced by several BDEs. That such an assumption is justified, and in line with the conservatisms required in risk assessment, is supported by the recent opinion of SCHER (2011) on the topic of PBDEs.

The analysis also shows that a consideration of BDE-209 in isolation, without taking account of co-exposure to several other BDEs, would grossly underestimate risks and create a false sense of security. This is all the more important since BDE-209 undergoes transformation reactions which liberate the more toxic lower brominated congeners, both through biotic processes in humans and in other organisms, as well as abiotic transformations in the environment.

6.2.8 References

EFSA 2011, "Scientific opinion on polybrominated diphenyl ethers (PBDEs) in food", EFSA Panel on Contaminants in the Food Chain (CONTAM), *EFSA Journal*, vol. 9, no. 5, pp. 2156.

Meek, M.E., Boobis, A.R., Crofton, K.M., Heinemeyer, G., Van Raaij, M., Vickers, C., 2011, "Risk assessment of combined exposure to multiple chemicals: A WHO/IPCS framework", *Regulatory Toxicology and Pharmacology*, vol 60, pp. S1-S14.

SCHER, Scientific Committee on Health and Environmental Risks, 2011, "Opinion on chemicals and the Water Framework Directive: Draft environmental quality standards PBDE", European Union ISSN 1831-4775, ISBN 978-92-79-30708-9, doi:10.2772/95861

6.3 Mixture risk assessment, environment

6.3.1 Aim and scope

This section explores options for conducting initial tier 0 screening level mixture risk assessments (MRA) for the sample set of 76 real-world multi-component exposure scenarios compiled in section 5.2.2 from PBDE exposure studies. The aim is to identify exposure situations that give reasons for concern and where future in-depth studies should consequently focus on, and to separate these from cases where adverse mixture effects appear unlikely to occur on the basis of all available data.

6.3.2 Applicable methodology and selection of congeners for inclusion in MRAs

All the component-based MRA methods that have been briefly described in section 6.1 have one thing in common: availability of individual toxicity data for all mixture components included in an assessment is an indispensable pre-requisite for their application. Unfortunately, this essential requirement cannot be fulfilled for the 76 exposure scenarios that are to be assessed. As detailed in section 5.2.2, the scenarios demonstrate situations of co-exposure to up to 46 different PBDE congeners out of a total of 70 congeners that were measured in one or more samples. In contrast, comparable eco-toxicity data are available for very few of the individual congeners and most of the eco-toxicity data refer to commercial PBDEs which are already mixtures in themselves (see section 5.2.3). In addition, the available data do not allow to identify a systematic rank order of toxicity for different congeners and ecotoxicological endpoints (with the possible exception of BDE-209, as detailed below). And furthermore, the available data also do not allow to identify any eco-toxicological modes of action that would be specific for specific groups of congeners. Thus there is no reliable basis for bridging the data gaps by read-across approaches, and there is also no good reasoning for limiting ecotoxicological MRAs to certain groups of PBDEs.

The only possible way out of this dilemma is to bridge all the gaps in eco-toxicity data by means of simple worst case assessments, assuming that the lowest observed effect concentration or no-observed effect concentration for an eco-toxicological endpoint applies to all PBDEs included in an MRA. For the purpose of a tier 0 screening level MRA, such a simple worst case approach can be transferred to the level of Hazard Index calculations (HI), as defined in section 6.1.2.1.1. This means that for a specific protection goal a uniform regulatory acceptable level (AL) may be defined for all congeners included in an MRA. In this case, the formula for the HI calculation reduces to a simple comparison of the sum of individual exposure levels (EL_i) of the co-occurring congeners with the uniform acceptable level (AL):

$$HI = \sum_{i=1}^{n} \frac{EL_i}{AL_i} = \frac{\sum_{i=1}^{n} EL_i}{AL}, \text{ if all } AL_i \text{ are equal}$$

Basically, this approach has been taken for the recent revision of environmental quality standards (EQS) for PBDEs under the European water framework directive (EU 2013). However, the new uniform EQS that were derived by the worst case approach do legally not apply to all PBDE congeners, but only to the sum of concentrations of a set of six selected indicator congeners that shall be included in all routine monitoring programs. These six indicator PBDEs are BDE-28, -47, -99, -100, -153, and -154. The confinement to these six indicator congeners was justified by considerations of analytical feasibility, production volumes and occurrence.

The confinement of the group EQS to the six indicator congeners was criticised by SCHER, the European Commission's Scientific Committee on Health and Environmental Risks. SCHER proposed to assume "*as a practical approach for the time being that all substances have the same mode of action and toxicity. This practical approach would mean that the total concentration of PBDEs (i.e. the sum of all individual PBDEs quantified in a sample) should be calculated and compared to the EQS*" (EC 2011c, p. 9). This proposal was not adopted in the legislation, but it was recognized in a background document "*that the consideration of only 6 congeners for monitoring may be underprotective if an additive mode of action is assumed for all 209 BDE congeners*" and it was "*noted that this would be the case in sampling sites where the six indicators are not the main contributors to the BDEs concentration*" (EC 2011b, p. 2).

The analyses of the sample set of 76 exposure scenarios from section 5.2.2 has clearly shown that it is not at all unusual to see under real environmental conditions that the six EQS indicator congeners do in fact not even account for half of the total PBDE concentrations found. Consequently, for an MRA that is not underprotective it is self-suggesting to follow the SCHER proposal and to compare total measured PBDE concentrations to uniform quality standards as reference values.

In doing so, some special considerations may be necessary regarding the inclusion or non-inclusion of BDE-209 in the calculations. As detailed in section 5.2.3, BDE-209 was often found to be non-toxic at water soluble concentrations. Hence it may be argued that BDE-209 does not contribute to a dose additive effect and that it should therefore not be included in comparisons of sums of PBDE concentrations with a uniform reference value that was derived from worst-case toxicity data. This may result in overly conservative MRAs. On the other hand, part of the available studies clearly demonstrated adverse effects at concentrations that may have an environmental relevance, and hence a non-inclusion may result in under-protective MRAs. Obviously, the answer to the question whether BDE-209 may contribute to an additive mixture effect depends on the specific species, endpoint, and exposure route. However, in general it can be stated that BDE-209 is less toxic or at least not more toxic than lower-brominated congeners. For an initial screening level MRA it is therefore self-suggesting to simply compare the consequences of the two possibly extreme assumptions: BDE-209 is either assumed to make no contribution to the mixture toxicity and left out of the calculation (potentially under-protective), or it is assumed to be as toxic as the most toxic congener and included in comparisons of the sum of PBDE concentrations with uniform worst-case based reference values (probably over-conservative). More specific considerations and refined calculations of mixture risk indicators may thereby be confined to situations where the results of the tier 0 assessment show that the inclusion or non-inclusion of BDE-209 is indeed crucial for the final MRA result in terms of exceedance or non-exceedance of a value of one for the HI.

In summary of these considerations, we always performed comparative calculations of HI values for every exposure scenario by including three different numbers of mixture components each:

(i) the 6 EQS indicator congeners only, assuming that they drive the overall risk;
(ii) all PBDEs except BDE-209, assuming that BDE-209 is non-toxic and does not contribute to the overall risk;
(iii) all PBDEs, as a worst case estimate on the basis of available data.

6.3.3 Reference values and selection of assessable exposure scenarios

6.3.3.1 Source of reference values

As reference values for assessing PBDE concentrations found in environmental samples, we used Specific Quality Standards (QS) that have been proposed by the Sub-Group on Review of the Priority Substances List under Working Group E of the Common Implementation Strategy for the Water Framework Directive, as explained in the following. The Specific QS and their derivation have been documented in a publicly available document (EC 2011), in the following shortly denoted as the PBDE EQS Dossier.

Based on the PBDE EQS Dossier, two types of Environmental Quality Standards (EQS) for PBDEs have recently been established in the European Union by Directive 2013/39/EU amending the Water Framework Directive 2000/60/EC and Directive 2008/105/EC on environmental quality standards in the field of water policy (EU 2013). These are

(i) an Annual Average EQS (AA-EQS) of 0.0085 μg/kg wet weight (ww) in biota, which relates to fish and which is for the protection of human health from adverse effects of PBDE contaminated food as the most critical endpoint, and
(ii) Maximum Allowable Concentrations (MAC-EQS) in fresh and marine waters of 0.14 and 0.014 μg/l, respectively, which aim to protect aquatic life from acute effects of peak exposures.

In addition to these legally binding EQS, however, the PBDE EQS Dossier also proposed so-called Specific Quality Standards (QS) for

(i) the protection of pelagic and benthic communities from chronic effects of direct exposure through water and sediment, respectively, and

(ii) the protection of predators from so-called secondary poisoning via PBDE contaminated biota that they use as a food source.

We used these Specific QS as reference values for a tier 0 screening level assessment of risks of PBDE mixtures to wildlife.

6.3.3.2 *Conversion factors*

In the PBDE EQS Dossier, the biota QS, i.e. the Specific QS for the prevention of secondary poisoning, is defined in terms of μg/kg ww of whole organisms. Many monitoring data, however, are expressed in terms of a lipid normalized concentration. For the comparison of such monitoring data with the biota QS, we assumed a standard lipid content of 5 %, as suggested in the Technical Guidance for Deriving Environmental Quality Standards (EC 2011a, p.76). This means that a lipid-based reference value was obtained by multiplying the biota QS with a conversion factor of 20.

Some of the monitoring data for biota are reported on a dry weight basis (dw). In these cases we assumed the moisture of the wet material to be 90 %, thereby following the example of a case study on aquatic predators given in the Technical Guidance for Deriving Environmental Quality Standards (EC 2011a, p.192). This means that a dry weight based reference value was obtained by multiplying the biota QS with a conversion factor of 10.

One of the examined monitoring studies (Bartrons et al. 2011) reported PBDE concentrations in microbial freshwater biofilms on an organic matter basis (OM). In this case, we assumed an average carbon content of 50 % of the ash-free dry weight (Wetzel 2001), and again 90 % moisture of the native material. This means that an OM-based reference value was obtained by multiplying the biota QS with a conversion factor of 20.

6.3.3.3 *Reference values applied*

Given the above considerations on applicable conversion factors, the Specific QS proposed in the PBDE EQS Dossier and used here as reference values for a screening level MRA of PBDEs are the following:

- 0.049 μg/l freshwater, for the protection of the pelagic community
- 0.0049 μg/l marine water, for the protection of the pelagic community
- 1.550 μg/kg dw in freshwater sediments, for the protection of the benthic community
- 310 μg/kg dw in marine sediments, for the protection of the benthic community
- 44 μg/kg ww or 880 μg/kg lw or 440 μg/kg dw or 880 μg/kg OM in biota for the protection of predators from secondary poisoning.

Under the WFD, the derivation of biota QS for the prevention of secondary poisoning is focussed on fish eating predators. However, the specific biota QS for PBDEs was derived from a rat toxicity study and may be considered to have a more general validity, not limited to fish as the food source. For the purpose of screening level MRAs, we used the proposed biota QS as a general reference value for assessing PBDE levels in all kinds of biota (including plants) for protecting all kinds of animals, not only “predators” in a narrow sense, but also including consumers (of plant materials) and omnivores.

6.3.3.4 *Assessable exposure scenarios*

With the reference values given above, hazard indices can be calculated for 65 out of the 76 exposure scenarios that were selected in section 5.2.2 from available multi-component monitoring studies. The 11 scenarios that cannot be compared to any of the reference values are the following:

(i) 2 scenarios on exposure levels defined in terms of PBDE concentrations measured in particulate material filtered out from freshwater samples (Zhang et al. 2010); no available reference values compares to such a dose metric or could be easily converted into a comparable measure.

(ii) 7 scenarios on exposure levels found in soil (Jiang et al. 2010, Li et al. 2011, Yang et al. 2008, Yu et al. 2011, Zhang et al. 2010) or in sewage sludge (Hwang et al. 2012) that may be applied to agricultural soils; available quality standards do not include the soil compartment.

(iii) 1 scenario on plasma concentrations of PBDEs in polar bears (Verreault et al. 2005); conversion into estimates of whole body concentrations and application of the biota QS is pointless for such a top predator; assessing the health risk for polar bears themselves is not possible on this basis, as no mammalian toxicity data are available that refer to internal blood plasma concentrations or which could be easily converted into comparable reference values.

6.3.4 Results

HI calculations for each of the 65 selected assessable exposure scenarios are compiled in Table 27. Information on exposure levels is taken from Table 15 (Section 5.2.2) with the only difference that concentrations are now uniformly expressed in dimensions of µg/l or µg/kg to be easily comparable with the applicable reference levels and with each other. Grouping of the exposure scenarios is partly revised for achieving full compliance with the applicable reference values and the protection goals for which they have been defined. As explained above, for every exposure scenario total exposure levels are given for the sum of (i) the 6 indicator congeners that apply for PBDE EQS in the EU (shortly denoted as the "EU EQS congeners"), (ii) the sum of all PBDEs except BDE-209, and (iii) the sum of all PBDES including BDE-209. These total exposure levels are divided by the applicable uniform reference values, resulting in the Hazard Indices reported in the table.

6.3.4.1 Direct exposure of pelagic and benthic communities

No significant mixture risks were detected for any of the sample scenarios of direct exposure of pelagic or benthic communities to PBDEs detected in the water phase or in sediments. Corresponding HI values are well below the trigger value of one, typically several orders of magnitude. Thus, any refinement of the tier 0 screening level MRA for these scenarios is worthless, unless new toxicity data should become available that indicate that previously untested species and/or endpoints are orders of magnitude more sensitive than the known ones.

6.3.4.2 Secondary poisoning

No significant mixture risks were also detected for most of the scenarios for secondary poisoning of consumers or predators via biota that they may use as a food source. Typically, HI values are higher than those seen for direct exposure scenarios for pelagic and benthic communities, but still not higher than the trigger value of one.

Exceptions to the rule were seen in a total of 11 exposure scenarios reported in a total of seven studies. For these scenarios the resulting HI values that are higher than one are highlighted in the table by a grey filling of the data cells. Most of these 11 scenarios for which the HI values indicate an exceedance of the reference values and hence a potential for adverse additive effects, reflect situations at local pollution hot spots or at least in highly industrialized areas, such as

- grass, haricot beans, and saline seepweed sampled in the surroundings of PBDE production plants in China (Jin et al. 2008),
- bivalves (Corbicula fluminea) and gastropods (Elimia proxima) collected downstream from a textile manufacturing outfall in the USA (La Guardia et al. 2012),
- two different samples of larval loaches kept in a net-cage at an e-wastes recycling site in China (Qin et al. 2009),
- adult frogs (Rana limnocharis) collected from a rice field in an e-waste recycling site in China (Liu et al. 2011),
- livers of ring-billed gulls breeding in a highly industrialized section of the St. Lawrence River, downstream from Montreal (QC, Canada) with both major point-sources and diffuse contamination (Gentes et al. 2012), and
- plankton samples from Lake Michigan, North America (Kuo et al 2010).

However, one of the 11 exposure scenarios with HI values above one gives reasons for the concern that high exposure levels above the applicable quality standards may also occur in remote areas. Shaw et al. (2008) reported relatively high PBDE levels in blubber of harbor seals (Phoca vitulina concolor) collected between 1991 and 2005 along the northwest Atlantics. The corresponding HI values calculated in Table 27 are 2.66 for the six EU EQS congeners and 2.77 for all PBDEs, whereby BDE-209 did not make a significant contribution. This finding provides evidence in support of the view that mammalian top-level predators feeding on seals, in particular polar bears, may be in danger of adverse effects that could result from the intake of PBDE mixtures via food. To clarify the issue, in-depth research work is required.

In all but two of the 11 critical cases, exceedance of the critical HI value of one was observed for any of the three comparative calculations, including either only the six EU EQS congeners, or all PBDES except BDE-209 or all PBDEs including BDE-209. Thus, these findings are very clear, even observed with the least conservative assumption possible.

Only in the remaining two scenarios did assumptions about the possible contributions of BDE-209 to the mixture toxicity turn out to be critical for the overall MRA. This applies to exposure situations seen in saline seepweed samples from a pollution hot spot in China (Jin et al. 2008) and in plankton samples from Lake Michigan, North America (Kuo et al 2010). In both cases, BDE-209 is the dominating component and HI values above one are calculated only under the worst case assumption that BDE-209 is as toxic as the most toxic lower brominated congener, i.e. total PBDE concentrations including BDE-209 are compared to the biota QS. Thus, in these two cases refined assessments of the actual toxicity of BDE-209 to organisms feeding on saline seepweed and Lake Michigan plankton, respectively, would be required for a refined MRA.

6.3.5 Conclusions

Results from a tier 0 screening level MRA for a sample set of 65 mixture exposure scenarios support the view that in general the likelihood of adverse effects that may result from exposure to PBDE mixtures is much lower for wildlife than for humans. Exceptions to the rule may not only apply to local hot spots of PBDE contamination from industrial activities, but also to mammalian top level predators in remote areas, such as polar bears in particular. To clarify the issue further, dedicated research work is necessary.

Table 27: Tier 0 screening level MRA for 65 mixture exposure scenarios from monitoring studies

Hazard Index values >1 indicate possible mixture risks that require clarification by refined assessments (highlighted in grey)

Exposure Matrix		Reference	No of congeners detected / targeted		Unit*	Sum of 6 EU EQS congeners	Sum of all except BDE-209	Sum of all
SAMPLE TYPE	Specification							
I. FRESHWATER COMPARTMENT								
Protection Goal: Pelagic community (freshwater)								
FRESHWATER (dissolved PBDE)		Zhang et al. 2010	11/14					
					Exposure Level	µg/l	1.77E-05	2.44E-05	9.14E-05
					Reference Level	µg/l	0.049	0.049	0.049
					Hazard Index		**0.00036**	**0.00050**	**0.00187**
FRESHWATER (dissolved PBDE)		Zhang et al. 2010	11/14					
					Exposure Level	µg/l	1.61E-05	2.21E-05	7.41E-05
					Reference Level	µg/l	0.049	0.049	0.049
					Hazard Index		**0.00033**	**0.00045**	**0.00151**
II. MARINE WATER COMPARTMENT								
Protection Goal: Pelagic community (marine)								
MARINE WATER		Moeller et al. 2012	3/10					
					Exposure Level	µg/l	2.35E-07	2.35E-07	1.09E-06
					Reference Level	µg/l	0.0049	0.0049	0.0049
					Hazard Index		**0.00005**	**0.00005**	**0.00022**
MARINE WATER		Moeller et al. 2012	6/10					
					Exposure Level	µg/l	5.05E-07	5.95E-07	5.25E-06
					Reference Level	µg/l	0.0049	0.0049	0.0049
					Hazard Index		**0.00010**	**0.00012**	**0.00107**

Table 27: Tier 0 screening level MRA for 65 mixture exposure scenarios from monitoring studies

Hazard Index values >1 indicate possible mixture risks that require clarification by refined assessments (highlighted in grey)

Exposure Matrix		Reference	No of congeners detected / targeted		Unit*	Sum of 6 EU EQS congeners	Sum of all except BDE-209	Sum of all
SAMPLE TYPE	Specification							
MARINE WATER		Moeller et al. 2012	6/10	Exposure Level	µg/l	1.22E-06	1.32E-06	3.47E-06
				Reference Level	µg/l	0.0049	0.0049	0.0049
				Hazard Index		**0.00025**	**0.00027**	**0.00071**
MARINE WATER		Moeller et al. 2011	5/10	Exposure Level	µg/l	3.22E-05	4.87E-05	5.30E-05
				Reference Level	µg/l	0.0049	0.0049	0.0049
				Hazard Index		**0.00657**	**0.00994**	**0.01081**

III. FRESHWATER SEDIMENT COMPARTMENT

Protection Goal: Benthic community (freshwater)

FRESHWATER SEDIMENT		Zhang et al. 2010	14/14	Exposure Level	µg/kg dw	0.1	1.2	17.3
				Reference Level	µg/kg dw	1550	1550	1550
				Hazard Index		**0.00004**	**0.00080**	**0.01117**
FRESHWATER SEDIMENT		Zhang et al. 2010	14/14	Exposure Level	µg/kg dw	0.1	1.3	9.8
				Reference Level	µg/kg dw	1550	1550	1550
				Hazard Index		**0.00005**	**0.00083**	**0.00633**

Table 27: Tier 0 screening level MRA for 65 mixture exposure scenarios from monitoring studies

Hazard Index values >1 indicate possible mixture risks that require clarification by refined assessments (highlighted in grey)

Exposure Matrix		Reference	No of congeners detected / targeted		Unit*	Sum of 6 EU EQS congeners	Sum of all except BDE-209	Sum of all
SAMPLE TYPE	Specification							
FRESHWATER SEDIMENT		Marvin et al. 2013	46/46	Exposure Level	µg/kg dw	13.6	19.1	46.5
				Reference Level	µg/kg dw	1550	1550	1550
				Hazard Index		**0.00878**	**0.01233**	**0.03001**
FRESHWATER SEDIMENT		Marvin et al. 2013	46/46	Exposure Level	µg/kg dw	34.7	48.1	107.2
				Reference Level	µg/kg dw	1550	1550	1550
				Hazard Index		**0.02236**	**0.03104**	**0.06917**

IV. MARINE SEDIMENT COMPARTMENT

Protection Goal: Benthic community (marine)

MARINE SEDIMENT		Shanmuganathan et al. 2011	18/23	Exposure Level	µg/kg dw	0.1	0.2	3.8
				Reference Level	µg/kg dw	310	310	310
				Hazard Index		**0.00018**	**0.00058**	**0.01220**
MARINE SEDIMENT		Shanmuganathan et al. 2011	18/23	Exposure Level	µg/kg dw	0.2	0.4	75.5
				Reference Level	µg/kg dw	310	310	310
				Hazard Index		**0.00069**	**0.00141**	**0.24366**

Table 27: Tier 0 screening level MRA for 65 mixture exposure scenarios from monitoring studies

Hazard Index values >1 indicate possible mixture risks that require clarification by refined assessments (highlighted in grey)

Exposure Matrix		Reference	No of congeners detected / targeted		Unit*	Sum of 6 EU EQS congeners	Sum of all except BDE-209	Sum of all
SAMPLE TYPE	Specification							
MARINE SEDIMENT		deBruyn et al. 2009	34/46	Exposure Level	µg/kg dw	2.9	3.8	6.4
				Reference Level	µg/kg dw	310	310	310
				Hazard Index		**0.00947**	**0.01229**	**0.02051**

V. BIOTA

Protection Goal: Consumers and Predators (secondary poisoning)

TERRESTRIAL PLANT	grass	Yu et al. 2011	10/16	Exposure Level	µg/kg dw	0.2	1.8	5.2
				Reference Level	µg/kg dw	440	440	440
				Hazard Index		**0.00046**	**0.00410**	**0.01182**
TERRESTRIAL PLANT	grass	Jin et al. 2008	11/11	Exposure Level	µg/kg ww	281.9	371.4	1,011.4
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**6.41**	**8.44**	**22.99**
TERRESTRIAL PLANT	haricot bean	Jin et al. 2008	10/11	Exposure Level	µg/kg ww	51.6	67.9	157.9
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**1.17**	**1.54**	**3.59**

Table 27: Tier 0 screening level MRA for 65 mixture exposure scenarios from monitoring studies

Hazard Index values >1 indicate possible mixture risks that require clarification by refined assessments (highlighted in grey)

Exposure Matrix		Reference	No of congeners detected / targeted		Unit*	Sum of 6 EU EQS congeners	Sum of all except BDE-209	Sum of all
SAMPLE TYPE	Specification							
TERRESTRIAL PLANT	leaves	Yang et al. 2008	36/37	Exposure Level	µg/kg dw	108.0	199.0	202.9
				Reference Level	µg/kg dw	440	440	440
				Hazard Index		**0.25**	**0.45**	**0.46**
TERRESTRIAL PLANT	saline seepweed	Jin et al. 2008	4/11	Exposure Level	µg/kg ww	0.0	18.7	70.7
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**0.00**	**0.43**	**1.61**
TERRESTRIAL PLANT	tree bark	Zhu and Hites 2006	13/13	Exposure Level	µg/kg lw	13.6	24.8	101.6
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.02**	**0.03**	**0.12**
AQUATIC PLANTS	sea weed / macroalgae	Shanmuganathan et al. 2011	20/23	Exposure Level	µg/kg dw	0.3	0.5	4.0
				Reference Level	µg/kg dw	440	440	440
				Hazard Index		**0.00069**	**0.00118**	**0.00913**
MICROORGANISM	algae / phytoplankton	Shanmuganathan et al. 2011	23/23	Exposure Level	µg/kg dw	1.8	4.9	48.3
				Reference Level	µg/kg dw	440	440	440
				Hazard Index		**0.0041**	**0.0111**	**0.1097**

Table 27: Tier 0 screening level MRA for 65 mixture exposure scenarios from monitoring studies

Hazard Index values >1 indicate possible mixture risks that require clarification by refined assessments (highlighted in grey)

Exposure Matrix		Reference	No of congeners detected / targeted		Unit*	Sum of 6 EU EQS congeners	Sum of all except BDE-209	Sum of all
SAMPLE TYPE	Specification							
MICROORGANISM	plankton	Kuo et al. 2010	6/6	Exposure Level	µg/kg lw	80.0	80.0	30,080.0
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.091**	**0.091**	**34.182**
MICROORGANISM	microbial biofilm	Bartrons et al. 2011	15/15	Exposure Level	µg/kg OM	0.1	0.4	0.6
				Reference Level	µg/kg OM	880	880	880
				Hazard Index		**0.00017**	**0.00046**	**0.00068**
MICROORGANISM	microbial biofilm	Bartrons et al. 2011	15/15	Exposure Level	µg/kg OM	2.1	3.2	21.2
				Reference Level	µg/kg OM	880	880	880
				Hazard Index		**0.0023**	**0.0036**	**0.0241**
INVERTEBRATE	bivalve (Corbicula fluminea)	La Guardia et al. 2012	14/20	Exposure Level	µg/kg lw	11,856.0	21,170.0	64,870.0
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**13.5**	**24.1**	**73.7**
INVERTEBRATE	caddisfly	Vigano et al. 2009	11/12	Exposure Level	µg/kg lw	317.5	327.5	652.5
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.36**	**0.37**	**0.74**

Table 27: Tier 0 screening level MRA for 65 mixture exposure scenarios from monitoring studies

Hazard Index values >1 indicate possible mixture risks that require clarification by refined assessments (highlighted in grey)

Exposure Matrix		Reference	No of congeners detected / targeted		Unit*	Sum of 6 EU EQS congeners	Sum of all except BDE-209	Sum of all
SAMPLE TYPE	Specification							
INVERTEBRATE	dragonfly	Yu et al. 2011	13/16	Exposure Level	µg/kg lw	8.2	32.2	53.2
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.009**	**0.037**	**0.060**
INVERTEBRATE	gammarid	Vigano et al. 2009	10/12	Exposure Level	µg/kg lw	287.5	305.0	615.0
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.33**	**0.35**	**0.70**
INVERTEBRATE	gastropod (Elimia proxima)	La Guardia et al. 2012	12/20	Exposure Level	µg/kg lw	22,414.0	24,459.0	47,159.0
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**25.5**	**27.8**	**53.6**
INVERTEBRATE	grasshopper	Yu et al. 2011	13/16	Exposure Level	µg/kg lw	1.9	30.6	70.6
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.002**	**0.035**	**0.080**
INVERTEBRATE	mussel	deBruyn et al. 2009	36/46	Exposure Level	µg/kg dw	86.1	92.0	97.3
				Reference Level	µg/kg dw	440	440	440
				Hazard Index		**0.20**	**0.21**	**0.22**

Table 27: Tier 0 screening level MRA for 65 mixture exposure scenarios from monitoring studies

Hazard Index values >1 indicate possible mixture risks that require clarification by refined assessments (highlighted in grey)

Exposure Matrix		Reference	No of congeners detected / targeted		Unit*	Sum of 6 EU EQS congeners	Sum of all except BDE-209	Sum of all
SAMPLE TYPE	Specification							
FISH	Bighead Carp	Zhang et al. 2010	14/14	Exposure Level	µg/kg lw	11.7	17.8	20.6
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.013**	**0.020**	**0.023**
FISH	Bighead Carp	Zhang et al. 2010	14/14	Exposure Level	µg/kg lw	9.0	12.1	13.2
				Reference Level	**µg/kg lw**	**880**	**880**	**880**
				Hazard Index		**0.010**	**0.014**	**0.015**
FISH	Bluntsnout Bream	Zhang et al. 2010	13/14	Exposure Level	µg/kg lw	6.7	10.1	10.9
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.008**	**0.011**	**0.012**
FISH	Common Mullet	Zhang et al. 2010	13/14	Exposure Level	µg/kg lw	4.2	5.3	5.9
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.0047**	**0.0060**	**0.0067**
FISH	crucian carp	Zhang et al. 2010	14/14	Exposure Level	µg/kg lw	21.1	27.6	30.3
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.024**	**0.031**	**0.034**

Table 27: Tier 0 screening level MRA for 65 mixture exposure scenarios from monitoring studies

Hazard Index values >1 indicate possible mixture risks that require clarification by refined assessments (highlighted in grey)

Exposure Matrix		Reference	No of congeners detected / targeted		Unit*	Sum of 6 EU EQS congeners	Sum of all except BDE-209	Sum of all
SAMPLE TYPE	Specification							
FISH	Grass Carp	Zhang et al. 2010	10/14	Exposure Level	µg/kg lw	4.9	6.0	6.0
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.0056**	**0.0069**	**0.0069**
FISH	Grass Carp	Zhang et al. 2010	10/14	Exposure Level	µg/kg lw	5.0	6.2	6.2
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.0057**	**0.0070**	**0.0070**
FISH	herring and sprat	Carlsson et al. 2011	5/11	Exposure Level	µg/kg ww	1.1	1.3	1.3
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**0.024**	**0.028**	**0.028**
FISH	Largemouth Bass	Zhang et al. 2010	10/14	Exposure Level	µg/kg lw	23.6	29.9	29.9
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.027**	**0.034**	**0.034**
FISH	Loach	Qin et al. 2009	8/39	Exposure Level	µg/kg ww	1.0	1.1	1.1
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**0.022**	**0.025**	**0.025**

Table 27: Tier 0 screening level MRA for 65 mixture exposure scenarios from monitoring studies

Hazard Index values >1 indicate possible mixture risks that require clarification by refined assessments (highlighted in grey)

Exposure Matrix		Reference	No of congeners detected / targeted		Unit*	Sum of 6 EU EQS congeners	Sum of all except BDE-209	Sum of all
SAMPLE TYPE	Specification							
FISH	Loach	Qin et al. 2009	10/39	Exposure Level	µg/kg lw	301.8	349.7	349.7
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.34**	**0.40**	**0.40**
FISH	Loach	Qin et al. 2009	27/39	Exposure Level	µg/kg lw	10,376.4	19,874.5	19,875.9
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**11.8**	**22.6**	**22.6**
FISH	Loach	Qin et al. 2009	26/39	Exposure Level	µg/kg ww	163.0	312.2	312.3
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**3.7**	**7.1**	**7.1**
FISH	Mud Carp	Zhang et al. 2010	12/14	Exposure Level	µg/kg lw	8.9	11.1	11.8
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.010**	**0.013**	**0.013**
FISH	Mud Carp	Zhang et al. 2010	12/14	Exposure Level	µg/kg lw	11.3	13.5	14.3
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.013**	**0.015**	**0.016**

Table 27: Tier 0 screening level MRA for 65 mixture exposure scenarios from monitoring studies

Hazard Index values >1 indicate possible mixture risks that require clarification by refined assessments (highlighted in grey)

Exposure Matrix		Reference	No of congeners detected / targeted		Unit*	Sum of 6 EU EQS congeners	Sum of all except BDE-209	Sum of all
SAMPLE TYPE	Specification							
FISH	Tilapia	Zhang et al. 2010	12/14	Exposure Level	µg/kg lw	10.1	12.7	13.7
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.011**	**0.014**	**0.016**
FISH	Tilapia	Zhang et al. 2010	14/14	Exposure Level	µg/kg lw	47.4	62.7	73.8
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.054**	**0.071**	**0.084**
AMPHIBIAN	frog	Liu et al. 2011	8/8	Exposure Level	µg/kg ww	26.3	27.1	27.7
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**0.60**	**0.62**	**0.63**
AMPHIBIAN	frog	Liu et al. 2011	8/8	Exposure Level	µg/kg ww	15.7	15.9	17.1
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**0.36**	**0.36**	**0.39**
AMPHIBIAN	frog	Liu et al. 2011	8/8	Exposure Level	µg/kg ww	24.8	25.5	26.2
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**0.56**	**0.58**	**0.60**

Table 27: Tier 0 screening level MRA for 65 mixture exposure scenarios from monitoring studies

Hazard Index values >1 indicate possible mixture risks that require clarification by refined assessments (highlighted in grey)

Exposure Matrix		Reference	No of congeners detected / targeted		Unit*	Sum of 6 EU EQS congeners	Sum of all except BDE-209	Sum of all
SAMPLE TYPE	Specification							
AMPHIBIAN	frog	Liu et al. 2011	8/8	Exposure Level	µg/kg ww	25.6	26.4	28.4
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**0.58**	**0.60**	**0.64**
AMPHIBIAN	frog	Liu et al. 2011	8/8	Exposure Level	µg/kg ww	133.6	136.6	141.1
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**3.04**	**3.10**	**3.21**
BIRD	common eider	Carlsson et al. 2011	3/11	Exposure Level	µg/kg ww	0.8	0.8	0.8
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**0.018**	**0.018**	**0.018**
BIRD	common kestrel	Yu et al. 2011	15/16	Exposure Level	µg/kg lw	65.9	262.9	359.9
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.075**	**0.299**	**0.409**
BIRD	Eurasian tree sparrow	Yu et al. 2011	15/16	Exposure Level	µg/kg lw	27.3	176.9	244.9
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.031**	**0.201**	**0.278**

Table 27: Tier 0 screening level MRA for 65 mixture exposure scenarios from monitoring studies

Hazard Index values >1 indicate possible mixture risks that require clarification by refined assessments (highlighted in grey)

Exposure Matrix		Reference	No of congeners detected / targeted		Unit*	Sum of 6 EU EQS congeners	Sum of all except BDE-209	Sum of all
SAMPLE TYPE	Specification							
BIRD	glaucous gull	Verreault et al. 2005	11/11	Exposure Level	µg/kg ww	19.8	20.2	20.3
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**0.45**	**0.46**	**0.46**
BIRD	glaucous gull	Verreault et al. 2005	11/11	Exposure Level	µg/kg ww	19.5	19.8	20.0
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**0.44**	**0.45**	**0.45**
BIRD	herring gull	Carlsson et al. 2011	5/11	Exposure Level	µg/kg ww	7.2	7.2	7.2
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**0.16**	**0.16**	**0.16**
BIRD	herring gull	Carlsson et al. 2011	8/11	Exposure Level	µg/kg ww	6.4	7.1	8.4
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**0.14**	**0.16**	**0.19**
BIRD	ring-billed gulls	Gentes et al. 2012	9/46	Exposure Level	µg/kg ww	17.0	19.4	26.4
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**0.39**	**0.44**	**0.60**

Table 27: Tier 0 screening level MRA for 65 mixture exposure scenarios from monitoring studies

Hazard Index values >1 indicate possible mixture risks that require clarification by refined assessments (highlighted in grey)

Exposure Matrix		Reference	No of congeners detected / targeted		Unit*	Sum of 6 EU EQS congeners	Sum of all except BDE-209	Sum of all
SAMPLE TYPE	Specification							
BIRD	ring-billed gulls	Gentes et al. 2012	20/46	Exposure Level	µg/kg ww	101.9	146.1	203.3
				Reference Level	µg/kg ww	44	44	44
				Hazard Index		**2.32**	**3.32**	**4.62**
BIRD	short-tailed shearwater (Puffinus tenuirostris)	Tanaka et al. 2013	31/49	Exposure Level	µg/kg lw	1.8	30.3	66.5
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.002**	**0.034**	**0.076**
RODENT	brown rat	Yu et al. 2011	15/16	Exposure Level	µg/kg lw	10.5	93.1	138.1
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**0.01**	**0.11**	**0.16**
SEA MAMMAL	seal	Shaw et al. 2008	25/25	Exposure Level	µg/kg lw	2,340.4	2,437.4	2,438.6
				Reference Level	µg/kg lw	880	880	880
				Hazard Index		**2.66**	**2.77**	**2.77**

* ww – wet weight, lw – lipid weight, dw – dry weight, OM – organic material

6.3.6 References

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7. Discussion

The mixture risk assessment case study for human exposures revealed concerns about exposures to PBDEs beyond tolerable levels, especially for young children. The outcome of this assessment is based on the following assumptions which will be discussed here:

- BDE-209 and other PBDEs are capable of producing combination effects for developmental neurotoxicity.
- The combination effects follow the dose addition principle and can be evaluated according to the Hazard Index (HI) approach.

There is no experimental evidence of combination effects between BDE-209 and other PBDEs, for endpoints relevant to developmental neurotoxicity. Nevertheless, there are good reasons to believe that such combination effects arise: First, BDE-47 and -99 act together synergistically to induce cytotoxicity in neuronal cells (Tagliaferri et al. 2010). Direct cytotoxicity to neuronal cells is considered to be one mechanism through which developmental neurotoxicity can arise. This observation therefore reveals a potential for combination effects among PBDEs. Second, BDE-209, its debromination products BDE-183, -203 and -206, as well as BDE-47, -99 and -153 induce developmental neurotoxicity in rodent models. Considering this common adverse effect pattern, it is highly likely that these PBDE congeners will produce combination effects. To put it in a different way, a disregard for combination effects among PBDEs, including BDE-209, is equivalent with the conjecture that in combination only one congener is active, while all others are toxicologically inert. Since all the above congeners are known to produce developmental neurotoxicity when administered as single compounds, this assumption is hard to justify and runs counter to the available evidence.

In common with the approaches elaborated by WHO / IPCS (Meek et al. 2011) and EFSA (2013) in the absence of evidence to the contrary it should be assumed that combination effects by PBDEs follow the dose addition principle. This is the basis for using the HI method for an assessment of possible combined effects.

As detailed by EFSA (2011), there are concerns about the exposure of young children to BDE-99 alone. These concerns become apparent even without taking consideration of combination effects. However, for an evaluation of other PBDEs, including BDE-209, -47, -99 and -153, a disregard for possible combination effects would not meet the standards of conservatism required in chemical risk assessment and would lead to significant underestimations of the true risks.

This is particularly pronounced in the case of BDE-209. Evaluated in isolation, there is currently a margin of exposure significantly higher than the margin of 100 that is normally required as a minimum. However, since BDE-209 liberates lower brominated congeners through abiotic and biotic processes, it acts as a slow release reservoir for these more toxic congeners which are produced by humans directly from BDE-209, or reach humans through ingestion of food and contact with other materials. These more toxic congeners are then highly likely to act together to produce combination effects. An evaluation of the risks associated with BDE-209 exposures that did not take account of these processes, would be artificial and would lead to significant underestimations of risks.

In the case of an ecotoxicological mixture risk assessment, similar considerations apply. Our study revealed a case for evaluating more thoroughly a scenarios involving predation of seals by polar bears. Because of a lack of data, it was not possible to realise this.

7.1 References

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8. Conclusion

There is good evidence that BDE-209 transformation yields lower brominated PBDE, both through abiotic and biotic processes. BDE-209 thus acts as a slow release reservoir for these more toxic congeners.

There is concern especially about young children who are not sufficiently protected against the combined effects of BDE-209, its biotransformation products, and other PBDEs which are in the environment as a result of exposures in the past.

There is also concern about top arctic predators such as polar bears.

Any toxicological assessment of BDE-209 in isolation, without taking account of combination effects with other congeners will significantly underestimate risks to human health and biota.

9. Appendices

This report is accompanied by a series of appendices and data files, which are listed here.

9.1 Appendices

9.1.1 Appendix A

Database report, each article is shown with its classification results. 985pp.

9.1.2 Appendix B

A list of reports considered in this project

9.1.3 Appendix C

PRISMA checklist for the reporting of systematic reviews.

9.2 Data files

Data files are provided in Excel spreadsheet format (.xlsx).

9.2.1 EXCELFILE ECO_1

File lists the congeners examined in 304 studies of environmental exposure

9.2.2 EXCEL FILE ECO_2

File lists the selection process selected for compilation of environmental exposure

9.2.3 EXCEL FILE ECO_3

File lists exposure data for 70 congeners in selected studies

9.2.4 EXCEL FILE HUMAN_1

File lists data used for human exposure assessment

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The Norwegian Environment Agency's primary tasks are to reduce greenhouse gas emissions, manage Norwegian nature, and prevent pollution.

We are under the Ministry of Climate and Environment and have over 700 employees at our two offices in Trondheim and Oslo and at the Norwegian Nature Inspectorate's more than sixty local offices.

Our principal functions include monitoring the state of the environment, conveying environment-related information, exercising authority, overseeing and guiding regional and municipal authorities, cooperating with relevant industry authorities, acting as an expert advisor, and assisting in international environmental efforts.