baua:
Bundesanstalt für Arbeitsschutz und Arbeitsmedizin
Federal Institute for Occupational Safety and Health

SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

1,1'-(isopropylidene)bis[3,5-dibromo-4-(2,3-dibromopropoxy)benzene]

TBBPA-DBPE
EC number 244-617-5; CAS RN 21850-44-2

1,1'-(isopropylidene)bis[3,5-dibromo-4-(2,3-dibromo-2-methylpropoxy)benzene]

TBBPA-DBMPE
EC number 306-832-3; CAS RN 97416-84-7

Evaluating Member State(s): Germany

Dated: 22 November 2021

Evaluating Member State Competent Authority

BAuA
Federal Institute for Occupational Safety and Health
Division 5 - Federal Office for Chemicals
Friedrich-Henkel-Weg 1-25
D-44149 Dortmund, Germany

Year of evaluation in CoRAP: 2017

Member State concluded the evaluation without any further need to ask more information from the registrants under Article 46(1) decision.

Further information on registered substances here:

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

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

The two Substances 1,1'-(isopropylidene)bis[3,5-dibromo-4-(2,3-dibromopropoxy)-benzene (EC number 244-617-5 - TBBPA-DBPE) and 1,1'-(isopropylidene)bis[3,5-dibromo-4-(2,3-dibromo-2-methylpropoxy)benzene (EC number 306-832-3 - TBBPA-DBMPE) were originally selected for substance evaluation in order to clarify concerns about suspected PBT (persistent, bioaccumulative, toxic) properties and potential endocrine disruptive properties for the environment.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

For TBBPA-DBPE, a decision on a testing proposal was issued on 3 March 2016 requiring a pre-natal developmental toxicity study (OECD TG 414).[2]

3. CONCLUSION OF SUBSTANCE EVALUATION

Table 1

CONCLUSION OF SUBSTANCE EVALUATION	
Conclusions	**Tick box**
Need for follow-up regulatory action at EU level	
Harmonised Classification and Labelling	
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
Currently no need for regulatory follow-up action at EU level	X

Based on the currently available information, no regulatory follow-up action is foreseen by the eMSCA. However, the necessity for action on these substances may need to be reassessed in case more information becomes available from the substance evaluation on TBBPA (EC number 201-236-9, CAS RN 79-94-7), a possible impurity and metabolite of both substances, with regard to its hazardous properties for the environment.

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

Not applicable.

[2] ECHA decision on a testing proposal for TBBPA-DBPE from 3 March 2016: https://echa.europa.eu/documents/10162/b0534237-6c39-7d7c-3068-a3729823b1ff

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Currently not applicable.

4.1.3. Restriction

Currently not applicable.

4.1.4. Other EU-wide regulatory risk management measures

Not applicable.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Based on the currently available information, the substances themselves do not fulfil properties which would make them eligible for SVHC identification. Depending on the composition of the technical substances and the outcome of the substance evaluation on tetrabromobisphenol A (EC number 201-236-9, "TBBPA")[3], a building block of both substances and potential impurity or metabolism product (in organisms under special conditions), the need to identify the two substances as SVHC based on their impurity profile may become necessary. Depending on the outcome of the substance evaluation of TBBPA, both substances may be regarded as precursors to a substance giving rise to concern for the environment, requiring further action.

Table 2

REASON FOR REMOVED CONCERN The concern could be removed because	
Clarification of hazard properties/exposure	X
Actions by the registrants to ensure safety, as reflected in the registration dossiers (e.g. change in supported uses, applied risk management measures, etc.)	

5.2. Other actions

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS (IF NECESSARY)

Not applicable.

[3] ECHA section on the substance evaluation of TBBPA: https://echa.europa.eu/de/information-on-chemicals/evaluation/community-rolling-action-plan/corap-table/-/dislist/details/0b0236e1807e837f

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

The two Substances 1,1'-(isopropylidene)bis[3,5-dibromo-4-(2,3-dibromopropoxy)-benzene (EC number 244-617-5 - TBBPA-DBPE) and 1,1'-(isopropylidene)bis[3,5-dibromo-4-(2,3-dibromo-2-methylpropoxy)benzene (EC number 306-832-3 - TBBPA-DBMPE) were originally selected for substance evaluation in order to clarify concerns about suspected PBT (persistent, bioaccumulative, toxic) properties and potential endocrine disruptive properties for the environment.

Both substances were assessed jointly as they are similar substances:

TBBPA-DBMPE has an additional methylgroup on each of the two alkyl chains which TBBPA-DBPE does not possess. A short table to elucidate read-across is in section 7.14. Both TBBPA-DBPE and TBBPA-DBMPE have the same potential metabolism product, TBBPA (the possibility for biotransformation under special conditions is depicted in section 7.10.1.2.2 Biotransformation). In summary, it is suggested that formation of TBBPA by metabolism of TBBPA-DBPE and TBBPA-DBMPE can occur under anaerobic conditions and in the presence of cyanocobalamin.

Table 3

EVALUATED ENDPOINTS FOR THE SUBSTANCES TBBPA-DBPE AND TBBPA-DBMPE	
Endpoint evaluated	**Outcome/conclusion**
Persistence	Concern confirmed. Based on available data from an OECD TG 307 simulation test on TBBPA-DBPE (which is used as a read-across substance for TBBPA-DBMPE), both substances are considered as very persistent in soil.
Bioaccumulation	Concern unresolved. The high hydrophobicity of the substances (log Kow > 10) raises a concern for slow bioaccumulation which however cannot be investigated further with existing validated methods. Based on the currently available PBT guidance (ECHA 2017) and the available data for TBBPA-DBPE, the eMSCA concludes that the B criterion for the two substances is likely not fulfilled.
Toxicity (T_{eco})	Concern refuted. Available ecotoxicity data on the substances did not show effects in fish, daphnia or algae. Hence, the eco-T criterion for the two substances is considered as not fulfilled. The T_{HH} criterion was not checked during the substance evaluation.
PBT/vPvB	Concern refuted. The substances fulfil the vP criterion according to Annex XIII of REACH. However, fulfilment of the (v)B and T criterion according to Annex XIII cannot be confirmed based on the existing data. Remaining concerns with regard to a slow bioaccumulation potential cannot be investigated further based on validated testing methods and PBT guidance. Hence, the substances are not regarded as PBT or vPvB by the eMSCA.
Endocrine disruption (ED) in the environment	Concern unresolved. The available *in vitro* tests on endocrine properties give indication for endocrine properties of the substance TBBPA-DBPE. However a conclusion is not possible, because the *in vitro* tests were conducted with the technical product with unknown purity. It is possible that the effects were caused by impurities like TBBPA or other endocrine acting substances. The available aquatic *in vivo* studies (see section 7.8.1) cannot release TBBPA-DBPE (or its impurities) from the suspicion of endocrine activity.

	The available *in vitro* tests on endocrine properties give indication for endocrine properties of the substance TBBPA-DBPE and hence for the read-across substance TBBPA-DBMPE.

7.2. Procedure

The formal evaluation process of the two substances was commenced in March 2017. The data from the registration dossiers were examined, as well as publicly available studies.

Following the initial evaluation, draft decisions with further information requirements were prepared by the eMSCA and sent to the registrants by ECHA for commenting. Based on the registrants' comments and all available information on the substances, the eMSCA terminated the decision making procedure and concluded the substance evaluation in 2021.

7.3. Identity of the substance

Table 2a

SUBSTANCE IDENTITY of TBBPA-DBPE	
Public name:	1,1'-(isopropylidene)bis[3,5-dibromo-4-(2,3-dibromopropoxy)benzene]
EC number:	244-617-5
CAS RN:	21850-44-2
Index number in Annex VI of the CLP Regulation:	N/A
Molecular formula:	$C_{21}H_{20}Br_8O_2$
Molecular weight range:	943.61 g/mol
Synonyms:	1,1'-propane-2,2-diylbis[3,5-dibromo-4-(2,3-dibromopropoxy)-benzene] Benzene, 1,1'-(1-methylethylidene)bis[3,5-dibromo-4-(2,3-dibromopropoxy)- 1,1'-(isopropylidene)bis[3,5-dibromo-4-(2,3-dibromopropoxy)benzene] 3,3',5,5'-Tetrabromobisphenol A bis(2,3-dibromopropyl) ether 4,4'-Isopropylidenebis[2,6-dibromo-1-(2,3-dibromopropoxy)benzene] Bis(2,3-dibromopropoxy)tetrabromobisphenol A TBBPA-DBPE Tetrabromobisphenol A 2,3-dibromopropyl ether Tetrabromobisphenol A bis(2,3-dibromopropyl ether)

Type of substance: Mono-constituent

Structural formula:

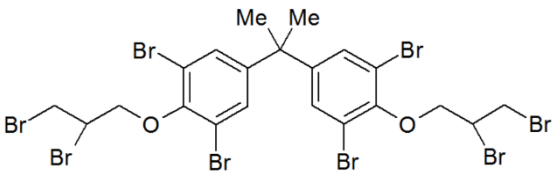
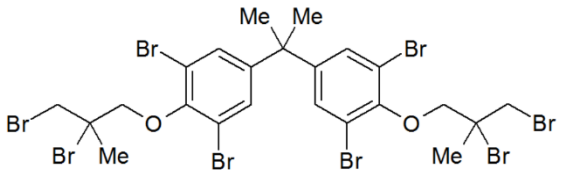


Table 3b

SUBSTANCE IDENTITY of TBBPA-DBMPE	
Public name:	1,1'-(isopropylidene)bis[3,5-dibromo-4-(2,3-dibromo-2-methylpropoxy)benzene]
EC number:	306-832-3
CAS RN:	97416-84-7
Index number in Annex VI of the CLP Regulation:	N/A
Molecular formula:	$C_{23}H_{24}Br_8O_2$
Molecular weight range:	971.7 g/mol
Synonyms:	▫ 1,1'-propane-2,2-diylbis[3,5-dibromo-4-(2,3-dibromo-2-methylpropoxy)benzene] ▫ 1,3-dibromo-2-(2,3-dibromo-2-methylpropoxy)-5-{2-[3,5-dibromo-4-(2,3-dibromo-2-methylpropoxy)phenyl]propan-2-yl}benzene ▫ Benzene, 1,1'-(1-methylethylidene)bis[3,5-dibromo-4-(2,3-dibromo-2-methylpropoxy)- ▫ AP 1300 S ▫ 1,1'-(1-Methylethylidene)bis[3,5-dibromo-4-(2,3-dibromo-2-methylpropoxy)benzene] ▫ 2,2-Bis[3,5-dibromo-4-(2,3-dibromo-2-methylpropoxy)phenyl]propane ▫ 2,2-Bis[3,5-dibromo-4-(2,3-dibromo-2-methylpropyloxy)phenyl]propane

Type of substance: Mono-constituent

Structural formula:



7.4. Physico-chemical properties

Table 3a

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES of TBBPA-DBPE	
Property	**Value**
Physical state at 20°C and 101.3 kPa	White crystalline powder
Melting point	113.39 °C [Differential scanning calorimeter]
Vapour pressure	0.029 Pa at 20°C [OECD TG 104 (Vapour Pressure Curve): static method]
Water solubility	0.144 µg/L at 20°C [OECD TG 105 (Water Solubility): column elution method]

Partition coefficient n-octanol/water (Log Kow)	log Pow >7.2 [OECD TG 123 (Partition Coefficient (1-Octanol /Water), Slow-Stirring Method)] 11.52 (estimated by KOWWIN v1.68)
Granulometry	10% of the volume distribution is below D10 = 12.534 µm; 50% of the volume distribution is below D50 = 44.819 µm; 90% of the volume distribution is below D 90 = 147.156 µm [Low Angle Laser Light Scattering (LALLS)]
Stability in organic solvents and identity of relevant degradation products	The stability of the substance is not considered to be critical. (There is no contact with organic solvents through the life cycle.)
Dissociation constant	n.a.

Table 4b

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES of TBBPA-DBMPE	
Property	**Value**
Physical state at 20°C and 101.3 kPa	White powder
Melting point	100-110 °C
Vapour pressure	n.a.
Water solubility	< 20 µg/L at 20 °C [OECD TG 105, flask method]
Partition coefficient n-octanol/water (Log Kow)	12.42 (calculation method)
Granulometry	10% of the volume distribution is below D10 = 1.97 µm; 50% of the volume distribution is below D50 = 7.99 µm; 90% of the volume distribution is below D 90 = 22.70 µm Laser light scattering
Stability in organic solvents and identity of relevant degradation products	The stability of the substance is not considered to be critical.
Dissociation constant	n.a. (acceptable data waiving)

7.5. Manufacture and uses

7.5.1. Quantities

Table 5a

AGGREGATED TONNAGE (PER YEAR) OF TBBPA-DBPE				
☐ 1 – 10 t	☐ 10 – 100 t	☐ 100 – 1000 t	☒ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

Table 5b

AGGREGATED TONNAGE (PER YEAR) OF TBBPA-DBPME				
☐ 1 – 10 t	☐ 10 – 100 t	☒ 100 – 1000 t	☐ 1000- 10,000 t	☐ 10,000-50,000 t
☐ 50,000 – 100,000 t	☐ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☐ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

Table 4a

USES for TBBPA-DBPE	Use(s)
Uses as intermediate	N/A
Formulation	The substance is used in closed processes during the preparation of polymers. However, since the substance is not covalently bound to the polymer matrix a continous release to man and environment during the article service life can be expected.
Uses at industrial sites	The environmental release categories are pointing to a possible wide dispersive exposure of the environment via these uses as a flame retardant in plastic articles.
Uses by professional workers	N/A
Consumer Uses	N/A
Article service life	The ERC provided by the registrants are ERC 10a and 11a pointing to wide dispersive outdoor and indoor use of long life plastic articles with low release. However, especially the wide dispersive outdoor use combined with the very high persistency of the substance raises exposure concern for the environmental compartments.

Table 5b

USES for TBBPA-DBPME	Use(s)
Uses as intermediate	N/A
Formulation	The substance is used in closed processes during the preparation of polymers. However, since the substance is not covalently bound to the polymer matrix a continous release to man and environment during the article service life can be expected.
Uses at industrial sites	The environmental release categories are pointing to a possible wide dispersive exposure of the environment via these uses as a flame retardant in plastic articles.
Uses by professional workers	N/A
Consumer Uses	N/A
Article service life	The ERC provided by the registrants are ERC 10a and 11a pointing to wide dispersive outdoor and indoor use of long life plastic articles with low release. However, especially the wide dispersive outdoor use combined with the very high persistency of the substance raises exposure concern for the environmental compartments.

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP) for both Substances

Neither TBBPA-DBPE nor TBBPA-DBMPE are listed in Annex VI CLP.

7.6.2. Self-classification for both Substances

Neither substance is self-classified in the registration dossiers.

No additional C&L notifications for both substances exist.

7.7. Environmental fate properties

7.7.1. Degradation

7.7.1.1 Degradation for TBBPA-DBPE

TBBPA-DBPE was not readily biodegradable in a screening test according to OECD TG 301 B[4].

To elucidate possible degradation pathways of TBBPA-DBPE the EAWAG Pathway Prediction system (PPS) was used. While some of the modelled degradation pathways of TBBPA-DBPE and its transformation products are likely, most are predicted to be neutral, i.e. the reactions are common but it is not certain that they occur in every system. Some neutral degradation pathways are predicted to result in 2,2,6,6-tetrabromo-4,4-isopropylidenediphenol (EC number 201-236-9, CAS RN 79-94-7, TBBPA).

A simulation study on degradation in soil according to OECD TG 307[5] does not confirm modelling, but shows TBBPA-DBPE to be very persistent in soil as ^{14}C- TBBPA-DBPE did not degrade under aerobic conditions in 120 days. Four different soils were used which differed in texture, organic carbon content, cation exchange capacity and humidity. Recovery rate predominantly was >97 %, temperature 20°C and the test lasted 120 days. No metabolites were detected and residual radioactivity was due to the parent substance ^{14}C- TBBPA-DBPE exempt for 3 % Non Extractable Residues. Hence, DT_{50} soil is >120 days.

Non-radiolabelled and radiolabelled (^{14}C-)TBBPA-DBPE did not degrade in a simulation study on degradation in water-sediment systems according to OECD TG 308 at 20°C in the dark for up to 100 days under anaerobic conditions. This results in a DT_{50} > 100 days. These findings are not unsual with respect on the aerobic results at hand and though results from anaerobic and aerobic tests cannot directly be compared they also do not question the assessment so far but confirm it.

7.7.1.2 Degradation for TBBPA-DBPME

In the registration data on TBBPA-DBMPE, a OECD TG 301B screening test on aerobic ready biodegradability is available. Based on the results (measured CO_2 evolution after 28 d test duration), the registrants conclude that TBBPA-DBMPE is not readily biodegradable. Further data on degradation of TBBPA-DBPME is not available, but due to its slightly more complex structure it is not probable that it is easier to degrade than TBBPA-DBPE. Thus reference is made to the degradation of TBBPA-DBPE except the modelling of degradation pathways which is presented for TBBPA-DBPME itself.

[4] Determination of the ready biodegradability of FR-720, Drake RM, 2002, Report No. ENV5608D/070151

[5] Aerobic degradation of TBBPA-DBPE in soil, Fraunhofer Institute for Molecular Biology and Applied Ecology, D-57392 Schmallenberg, Germany, Hennecke D., 20.6.2005, Report No. RIV-0001/7-90

7.7.2. Environmental distribution - Bioaccumulation

7.7.2.1. Bioaccumulation for TBBPA-DBPE

Screening criterion

Log K_{ow}

TBBPA-DBPE has an experimental log K_{ow} of > 7.2 (OECD TG 123). Estimated log K_{ow} values ranged between 10.4 (ACD Labs) and 11.5 (EPI SUITE). The documentation of this QSAR result does not comply with REACH Annex XI and thus its reliability is limited. In summary, a log K_{ow} > 10 is expected for TBBPA-DBPE. As the log K_{ow} is > 4.5, the screening criterion for bioaccumulation is fulfilled.

Indicators for limited bioaccumulation

Based on the fulfilled indicator for limited bioaccumulation (Dmax aver > 1.7 nm & molecular weight > 700 (1100) g/mol: criterion is fulfilled as the Dmax aver is 1.918 nm and the molecular weight is 943.6 g/mol; Log Kow > 10: The criterion is fulfilled as the expected log Kow of TBBPA-DBPE is > 10) high bioaccumulation potential of TBBPA-DBPE seems to be unlikely. Monitoring data and laboratory studies (e.g. Nyholm et al., 2008 & 2009 see below), however, show that TBBPA-DBPE can be taken up into organisms.

Experimental Data on aquatic Bioaccumulation

BCF

An OECD TG 305 test with *Cyprinus carpio* and aqueous exposure is available in the registration data for TBBPA-DBPE showing BCF (bioconcentration factor) values between 3.4 and 43 L/kg wwt (not lipid-normalized) for the high test concentration of 15 µg/l. For the low test concentration of 1.5 µg/l the BCF values ranged between 17 and 130 L/kg wwt (not lipid-normalized). The used test concentrations were 10 and 100 times higher than the water solubility of TBBPA-DBPE (0.144 µg/L). Therefore, the test is considered as not reliable by the eMSCA.

BAF

A sediment prolonged bioaccumulation study with *Hyalella azteca* is available for TBBPA-DBPE (RIVM, 2005a). The results are BAF (bioaccumulation factor) values <3 if related to sediment and BAF values around 70 if related to poor water. The authors assume that the test item was mostly adsorbed to the cuticula and not bioaccumulated in the amphipods. In summary, it was concluded that an ecologiccaly critical bioaccumulation of the test item in *Hyalella azteca* was not observed.

A sediment-water chironomid (*Chironomus riparius*) bioaccumulation test using spiked sediment is available for TBBPA-DBPE (RIVM, 2005b). The results are BAF < 1 if related to sediment, BAF between 12 and 46 if related to poor water and BAF between 71 and 103 if related to the overlaying water. The authors assume that the test item was adsorbed to the cuticula and not bioaccumulated in the larvae. In summary, it was concluded that an ecologically critical bioaccumulation of the test item in *Chironomus* larvae was not observed.

BMF

In a BMF (biomagnification factor) study by Nyholm et al. (2009) with zebrafish (*Danio rerio*) a mixture of 11 brominated flame retardants (BFRs), among them TBBPA-DBPE, was tested. Most of the substances showed a low recovery and thus it seems inappropriate to draw conclusions on uptake or elimination rates or metabolism. TBBPA-DBPE levels in zebrafish (*Danio rerio*) were not quantified because of the low recoveries in the spiked samples. The measured levels were well above the detection limit during exposure period of 42 d and below detection limit during the elimination period of 14 d. Due to experimental challenges the study provides no BMF for TBBPA-DBPE.

Further Data Regarding Aquatic Bioaccumulation

Transfer from fish to eggs (Nyholm et al., 2008)

In a feeding study with zebrafish (*Danio rerio*) similar to that described above (Nyholm et. al, 2009) eggs were collected directly after spawning. Possible contamination of eggs by feed and feces was minimized. TBBPA-DBPE was detected in all egg samples except on day 0. The lipid adjusted concentrations in fish were similar to the concentrations in eggs and the egg/fish concentration was significantly > 1. The study shows that TBBPA-DBPE was taken up by fish from food and was transferred to their eggs.

Mesocosm study (Jourdan et al., 2014)

The test with fathed minnow was conducted under semi natural conditions in outdoor mesocosms. Treatment concentration was chosen at 500 ng/g sediment in the upper 5 cm by using subsurface injection. The reported purity of the TBBPA-DBPE was >98%. The identity of the impurities was not determined. The sediment was not directly spiked (e.g., mixed with TBBPA-DBPE then added to the mesocosms), instead the BFRs were introduced into the mesocosms below the surface of the water and then they would naturally partition into the sediment (static system). The fathead minnows were added to the mesocosms prior to the addition of the BFRs to serve as an acclimation period. Fish were contained in mesh enclosures with 12 fish per enclosure and 2 enclosures per mesocosm. Plastic trays with sediment were added to the mesocosm to facilitate the removal and sampling of the sediment with minimal disturbance or resuspension of the sediments into the water column. A food grade plastic container with sediment was added to the mesh enclosures which housed the fish to provide habitat/shelter, to put the fish into closer contact with the sediment (the mesocosms were 1 m deep and the mesh enclosures are just below the surface) and to attract food sources. The fish were not fed but subsisted on the native zooplankton community. After 42 d uptake phase, fishes were transferred to the control mesocosms for the depuration phase (28 d). At day 7, 14, 28, 42, 49 and 70, fish were sampled. Each fish was collected into clean water prior to being placed in a bath of MS-222 – each of these steps may have reduced some surface binding. Further each organism was then blotted dry with skim wipes, removing moisture and potentially any surface bound contaminants including sediment. Whole body lipid extracts (minus plasma and liver) were analyzed for TBBPA-DBPE concentration. Measured concentration were given in ng/g lipid.

Growth dilution-adjusted concentrations of TBBPA-DBPE in whole-body extracts of minnows ranged from 6649 to 178 000 ng/g lipid, reaching the maximum concentration at day 14. No statistically significant decrease was observed during the depuration period and no statistically significant difference was observed between day 7 and day 70 ($\rho = 0.061$). The authors suspect that some of the pattern can be explained by the single treatment that the mesocosms received rather than continually dosing as seen in traditional uptake/kinetic studies.

Terrestrial Bioaccumulation for TBBPA-DBPE

In a test with terrestrial oligochaetes (*Eisenia fetida*, RIVM - Rijksinstituut voor Volksgezondheid en Milieu, 2005c), adult earthworms were placed in a defined artificial soil substrate containing the test item in two concentrations (2 mg/kg, 10 mg/kg). The concentration of TBBPA-DBPE in earthworms was found to be 0.04 mg/kg and 0.21 mg/kg fresh weight after 21 days of incubation in the 2 mg/kg and 10 mg/kg dry mass assays. The amounts are the residues of the contaminated soil in the worm gut after gut defaecation. There was only 0.01 mg/kg biomass measurable after 1 day of elimination.

7.7.2.2. Bioaccumulation for TBBPA-DBPME

Screening criterion

Log K_{ow}

For TBBPA-DBPME no experimental log Kow is available. For the read across substance TBBPA-DBPE an experimental Log Kow of > 7.2 (OECD TG 123) is available. Estimated log Kow values for TBBPA-DBMPE ranged between 11.1 (ACD Labs) and 12.4 (EPI SUITE). The documentation of this QSAR result does not comply with REACH Annex XI and thus its

reliability is limited. In summary, a log Kow > 10 is expected for TBBPA-DBMPE. As the log Kow is > 4.5 the screening criterion for bioaccumulation is fulfilled.

Indicators for limited bioaccumulation

Based on the fulfilled indicator for limited bioaccumulation (Dmax aver > 1.7 nm & molecular weight > 700 (1100) g/mol: criterion is fulfilled as the Dmax aver is 1.982 nm and the molecular weight is 971.6 g/mol; Log Kow > 10: The criterion is fulfilled as the expected log Kow of TBBPA-DBMPE is > 10) high bioaccumulation potential of TBBPA-DBMPE seems to be unlikely.

Experimental Data

No experimental data are availaible for TBBPA-DBMPE. Therefore, the experimental data of the read across substance TBBPA-DBPE were considered in the assessment for TBBPA-DBMPE.

Terrestrial Bioaccumulation for TBBPA-DBMPE

In a test with terrestrial oligochaetes (*Eisenia fetida*, RIVM 2005c) adult earthworms were placed in a defined artificial soil substrate containing the test item in two concentrations (2 mg/kg, 10 mg/kg). The concentration of TBBPA-DBPE in earthworms was found to be 0.04 mg/kg and 0.21 mg/kg fresh weight after 21 days of incubation in the 2 mg/kg and 10 mg/kg dry mass assays. The amounts are the residues of the contaminated soil in the worm gut after gut defaecation. There was only 0.01 mg/kg biomass measurable after 1 day of elimination.

Summary and Discussion of Bioaccumulation for TBBPA-DBPE and TBBPA-DBMPE

Based on the logK_{ow}, both substances screen as potential B/vB. The indicators for limited bioaccumulation are fulfilled and subsequently point to a low bioaccumulation potential. The available BCF study with carp is not reliable. Available BCF studies for TBBPA-DBPE with earthworm, amphipods and non-biting midge indicate surface sorption instead of uptake into the organisms. Nevertheless, available monitoring data and laboratory studies indicate that TBBPA-DBPE can be taken up into organisms. As the substance is highly hydrophobic (Log Kow > 10) a very slow uptake and clearence kinetic can be expected and reaching the steady state concentration can last years (Larisch and Goss, 2018). Subsequently, there is some concern for slow bioaccumulation. However, this is not covered by the current guidance and is therefore a development issue where more research is needed.

Based on the actual available guidance R.11 (2017) and the available data for both substances, the eMSCA concludes that it seems unlikely that TBBPA-DBPE or TBBPA-DBMPE are bioaccumulative in organisms.

7.8. Environmental hazard assessment for both Substances

7.8.1. Aquatic compartment (including sediment) for TBBPA-DBPE

7.8.1.1. Fish

Acute toxicity to fish

An acute fish toxicity test on TBBPA-DBPE was conducted with *O. mykiss* at a concentration of 100mg/L (water accommodated fraction (WAF), prepared by stirring for ten days, limit test) (TL, 2002a). For this test the solubility in water was determined to be in the range of 0.016-0.022 mg/l (deviating from the solubility in water of 0.14 µg/L, see Table 3). No vehicle was used. No effect was seen after 96h exposure (LC_{50} >100 mg/L (nominal)). The reliability of the assay was assessed to be Klimisch 1 by the registrants.

In another test (Yakata, 2003), TBBPA-DBPE was examined with *O. latipes*. Acetone (later evaporated) was used as vehicle here and HCO-40 (hydrogenated castor oil, ten times the amount of the test substance) for solution of the test substance. The test system was semi-static with a nominal concentration of 500 mg/L (The higher concentration of 5 000 mg/L was not used due to high concentration of dispersant). A high and a low exposure level of

15 and 1.5 µg/L was mentioned (in contradiction to the statement that the test at the higher concentration was not performed, hence only one concentration was tested). However, as no effects occurred it was not further considered. No effects were seen after 96 h (LC_{50} > 500 mg/L (nominal)).

Other studies with fish

Two mesocosm studies with *P. promelas* using technical TBBPA-DBPE (test substance FR-720) were conducted in 2008 and 2009 (de Jourdan, 2012). The mesocosm had a volume of 12 000 L, a depth of 1.2 m and a diameter of 3.9 m. In 2008 the mesocosm were performed with three replicates, but measurement of concentration was not conducted in 2008. In general, the tests in 2008 and 2009 were conducted in the same way aside from the number of replicates and the analysis. A solvent control existed (solvents DMSO - dimethylsulfoxide and toluene, 25:1, 0.001% solvent v/v). The total mass of TBBPA-DBPE per mesocosm was 350mg.

Second study in 2009: Fish were held in the mesocsom in separate cages (22 cm diameter, 40 cm long) with 12 fish per cage. 24 fish per mesocosm were used, with two replicates. The cage contained the same sediment like in the surrounding mesocosm: organic rich soil (1:1:1 mixture of topsoil:manure:compost) in a plastic container (10 x 10 x 5 cm). The exposure duration was 42 d, afterwards a 28 d depuration period was performed. The fish were not fed, but subsisted on the existing zooplancton.
The concentrations were measured in sediment and in the particulative phase only. In sediment the concentration was 0.00235 ng/g OC (mean of two ponds, study 2009); the concentrations in particulative phase: 0.454 and 0.556 ng/g OC (two ponds). Tissue and serum samples were taken to measure gonadosomatic index (GSI), vitellogenin (VTG), sex steroid production and accumulation of BFRs.

Results: No significant effects on VTG, estradiol (E2) and 11-ketotestosterone (11-KT) were seen. However the sample size was very small (two samples) and the standard deviation was very high. No effects on growth (three fish were sampled respectively at 7 d, 14 d, 28 d, and 42 d and during the depuration period at 49 d and 70 d), liver-somatic index (LSI) and GSI were seen.

Table 7

FISH TOXICITY TESTS					
Species	**Substance tested**	**Duration / Concentration / test condition**	**Result**	**Comments**	**Reference**
Oryzias mykiss	TBBPA-DBPE	96 h 100 mg/L (n)	LC_{50} > 100 mg/L (WAF)		(TL, 2002a)
Oryzias latipes	TBBPA-DBPE	96 h Vehicle Acetone, semi-static, Exposure: 15µg/L and 1.5µg/L (n)	LC_{50} > 500 mg/L (n)		(Yakata, 2003)
Pimephales promelas	Technical TBBPA-DBPE	In sediment: 0.00235 ng/g OC (mean of two ponds, study 2009); Concentration in particulate phase: 0.454 and 0.556 ng/g OC (two ponds) Vehicle: DMSO and Toluene (ratio 25:1, 0.001% solvent v/v) 42 day exposure + 28 d depuration; 24 fish per pond in two mesh enclosures; altogether 48 fish in two ponds, length of fish ca. 5cm (study 2009)	No significant effects on VTG, E2 and 11-KT (very small sample size and very high standard deviation). No effects on growth, LSI (number of sampled fish unknown)	Mesocosm study Endpoints: growth rate, condition factor, LSI, GSI, VTG and E2 and 11-KT in plasma. Male, female and immature fish were analysed separately.	(de Jourdan, 2012)
Danio rerio	TBBPA-DBPE (purity: no data)	Short term reproduction assay 21 d for adults, prolonged for offspring (144hpf), Semi-static, exposure via diet: 1, 10, 100 nmol/g dry weight (0.944, 9.44, 94.4µg/g dw) 8 males and 4 females per aquaria, two replicates, 40 eggs were studied per aquaria	No effects	Added endpoints: fertilization success, Embryos: development, hatching success, and survival	(Norman Haldén, 2010)
Danio rerio	TBBPA-DBPE (purity: no data)	FET: Embryo tox. Test, according to OECD Draft 2006 (FET); 144 hpf, Vehicle: DMSO (0.1%), tested in six concentrations (spacing factor 2.0 – 2.2), 24 fertilized eggs per treatment	No effects		(Norman Haldén, 2010)
Danio rerio	TBBPA-DBPE, purity 95%, (TCI Americas)	FET: Zebrafish embryos, exposure from 6 to 120 hpf; 6.4, 0.64, 0.064, 0.0064, 0.00064 µM Purity: 95%; Vehicle: DMSO (0.64%)	No significant effects on Mortality and Teratogenicity at 120 hpf. No hyper – or hypoactivity.	At 5 hpf embryos were enzymatically dechorionated.	(Noyes et al., 2015)

A Fish Short Term Reproduction Assay was conducted as part of a doctoral thesis according to OECD TG 229 (modified) with *D. rerio* examining several BFRs. (Norman Haldén, 2010) The guideline was modified with additional endpoints, prolonged exposure period and exposure via the diet. The study was conducted with exposure to control feed or feed contaminated with TBBPA-DBPE at nominal concentrations of 1, 10, and 100 nmol / g dry weight (0.944, 9.44, 94.4µg/g dw). For TBBPA-DBPE, no information about purity or another (trade) name was given. The exposure period was 21 days for adults. Each aquarium contained 12 adult zebrafish (approximately 8 males and 4 females). Two replicate aquaria were used per concentration. For prolonged exposure for offspring (144 hpf) 40 eggs per aquaria were studied.

To ensure that all feed was consumed and to minimize the risk of water contamination, fish were fed half of the dose in the morning and the other half in the afternoon. All fish were also fed brine shrimp as additional nutrition. Added endpoints were fertilization success and offspring viability, i.e. embryo development, hatching success and survival. The exposure period was preceded by a period, which lasted between one and three weeks (not specified), during which all groups were fed with control feed. The test condition was semi-static with one-third water exchanged at seven days per week. Feces and eggs were removed from the bottom of the aquaria at each water exchange. VTG was not determined in this assay.

Results: No effects were seen on fertilisation success, embryo development, hatching success and survival.

In the doctoral thesis, also a fish embryo toxicity test (FET) according to OECD Draft 2006 (FET) with *D. rerio* embryos was conducted (Norman Haldén, 2010). Eggs from unexposed fish were collected and exposed to TBBPA-DBPE. DMSO (0.1%) was used as vehicle. The fish embryos were tested in six concentrations (spacing factor 2.0 – 2.2, concentrations were not specified) with 24 fertilized eggs per treatment. Embryos were examined under a stereo microscope at 24, 48 and 144 hpf. Mortality was defined as lack of heart beat or coagulation. No effects were seen for TBBPA-DBPE. The author declared that the most hydrophic BFRs, among them TBBPA-DBPE, were not detected in the water and therefore could not be adequately assessed in the embryo toxicity test. Effects were caused by BFRs with log Kow between 4 and 7 (log Kow of TBBPA-DBPE > 7.2).

Another FET was conducted by Noyes et al. using TBBPA-DBPE with a purity of 95% (Noyes et al., 2015). A vehicle was used: DMSO (0.64%). At 5 hpf embryos were enzymatically dechorionated using pronase (enzym). Embryos were visually inspected under a light microscope after dechorionation and robotic plating to ensure embryo viability and proper staging. The zebrafish embryos were exposed from 6 to 120 hpf at the concentrations: 6.4, 0.64, 0.064, 0.0064, 0.00064 µM. No effects on mortality and teratogenicity were seen at 120 hpf. No hyper – or hypoactivity was observed.

7.8.1.2. Aquatic invertebrates

Daphnia magna acute toxicity test

A *Daphnia magna* acute toxicity test (TL, 2002c) according OECD TG 202 with TBBPA-DBPE was performed as a WAF test with limit concentration of 100 mg/L. The limit test consisted of 8 replicates for test concentration and controls and the exposure lasted 48 h. The test was static.

It was not possible to detect the test substance in the test media. Therefore, the nominal concentration of 100mg/L was given as the effect concentration EC_{50} > 100 mg/L WAF. 5% of *Daphnia* exposed to TBBPA-DBPE and none of the control Daphnia were immobilised. No *Daphnia* were trapped at the surface of water in control or test concentration.

A long term toxicity test for invertebrates is not available.

Table 8

AQUATIC INVERTEBRATES TOXICITY TESTS					
Species	**Substance tested**	**Duration / Concentration / test condition**	**Result**	**Comments**	**Reference**
D. magna	TBBPA-DBMPE	48 h 100 mg/L (n)	EC50 > 10 µg/L (n)	Measured concentrations were 81 to 109 % of nominal	(TL, 2015)
D. magna	TBBPA-DBPE	48 h 100 mg/L (n)	EC50 > 100 mg/L (WAF)	Japanese industrial standard	(TL, 2002c)

7.8.1.3. Algae and aquatic plants

Algae toxicity test:

The registration dossier for TBBPA-DBPE contains an algae toxicity test (using *Pseudokirchneriella subcapitata* formerly known as *Selenastrum capricornutum*) according to OECD TG 201 (TL, 2002b). The test was conducted with the test substance TBBPA-DBPE (FR-720 which is another name for TBBPA-DBPE). The purity was not specified. Two WAF concentrations (10 and 100 mg/L) were used. For controls, six replicates and for the two treatments three replicates existed. Analytical measurement of TBBPA-DBPE in the test medium was possible. The measured concentrations in the beginning of the test as described in the IUCLID (International Uniform Chemical Information Database) are unclear (it seems that for nominal 10 µg/L at t = 0 h the measured concentration was zero, after 72 h however the concentration was 0.002 mg/L; for nominal 100 mg/L at t = 0 h the measured concentration was 0.018 mg/L, and after 72 h 0.006 mg/L). The concentrations were not calculated due to this uncertainty. The test duration was 72 h. The EC_{50} (Half maximal effective concentration) values for growth and biomass are > 100mg/L (WAF). A NOEC was not derived in the registration.

Using the raw data effect values were calculated by the eMSCA. An effect of 7% was seen at the highest testing concentration and a significant lower cell density was determined. However, as the NOEC was in the range of normal limit of variation a new test is not required in the eyes of the eMSCA and the NOEC is not used for evaluation.

Table 9

ALGAE TOXICITY TEST					
Species	**Substance tested**	**Duration / Concentration / test condition**	**Result**	**Comments**	**Reference**
P. subcapitata	TBBPA-DBPE (FR-720)	72 h six replicates for controls and three replicates for treatments	ErC_{50} > 100 mg/L (WAF) EbC_{50} > 100 mg/L (WAF)	NOEC (see above in text)	(TL, 2002b)

7.8.1.4. Sediment organisms

The effect of technical TBBPA-DBPE on *Hyalella azteca* and the benthic macroinvertebrate community was assessed through the use of in situ exposure and sampling techniques in a mesocosm study (de Jourdan, 2012). The *in situ Hyalella* cages showed a high degree of variability for most endpoints, regardless of their placement (e.g., water column vs. sediment) in the mesocosm. There was very high variability within all the individual taxa, making it difficult to determine any statistical differences. No effects were seen. Due to the inconsistent results the test was not used for PNEC derivation.

Another toxicity test with TBBPA-DBPE was conducted according to US EPA guideline OPPTS (Office of Pollution Prevention and Toxics) 850.1735 (draft 1996) (TL, 2005b): Sediment prolonged toxicity study with *Hyalella azteca*, duration 28 d. It was used a sediment water system with defined artificial sediment and flow-through conditions. The sediment was spiked with the nominal test concentrations 30, 90, 270, 810, 2430 mg/kg dry sediment.

Two test items were used (test item A and test item B). Test item A (common name PE-68) with a purity of 99% was not radio labelled, test item B was ^{14}C radio labelled in the aromatic ring (purity > 99%). The animals were exposed over spiked sediment (Hyalella were fed with unspiked food). In the US EPA guideline OPPTS 850.1735 the exposure over contaminated food is not mentioned. However according to ECHA guidance R.10.5.2.2 feeding with spiked food is recommended for highly adsorptive substances. Feeding with unspiked food can possibly reduce exposure (to the test substance) via sediment ingestion. Eight replicates per treatments and control were used with 10 organisms per vessel. Age of organisms: 7 to 8 days. TBBPA-DBPE was dissolved in the solvent dichloromethane and mixed with quartz sand to obtain the required concentration. Then the solvent was evaporated. The same amount of dichloromethane/sand (dichloromethane evaporated) was also added to the control.
For verification of test substance concentration the radioactivity was measured by liquid scintillation counting in the sediment (after combustion) and in the water phase (without combustion). The concentration in the sediment was between 71 and 84% (mean measured activity). No or minor activity was measured in the pore water and overlying water. The concentrations in sediment were based on measured concentrations: 22.7, 71.6, 209, 642, 1837 mg/kg.

Results:
Length: A NOEC of 642 mg/kg was calculated using Williams Multiple t-test.
Weight: No significant effect was seen.
Survival: At two middle concentrations (71.6 and 209 mg/kg) the survival was significantly lower than control. However at the two highest concentrations no significant difference to control could be seen. Therefore the NOEC was assumed to be ≥ 1837 mg/kg.
For PNEC derivation the NOEC of 642 mg/kg sediment (reduced length) is used.

The authors of the mesocosm study also conducted a stand-alone test with *Chironomus dilutes*, aside from the mesocosm tests above (de Jourdan, 2012). The test was performed similar to standard procedure developed by the U.S. Environmental Protection Agency (USEPA) OPPTS 850.1735 (modified). As the test is also part of the doctoral thesis which described the mesocosm study above, it is most likely, that also technical TBBPA-DBPE (the commercial obtainable product FR-720 which does not consist other components according to the registrant's website) as test substance was used, no other information is available.
First instar larvae of *Chironomus dilutes* with an age at test start of 10-12 days after hatching, were exposed for 10 days. Sediment was spiked with TBBPA-DBPE at concentrations of 90, 900, 9 000 ng/g wet sediment. For the solvent control and the negative control and the three concentrations 6 replicates existed each. As solvent a mixture was used: iso-propanol (250 mL) and toluene (5 mL) on 1.5 L sediment.
Exposure chambers (300 mL) were filled with 100 mL of test sediment and approximately 100 mL of reconstituted water. The test was static with no renewal of water except for the replacement of overlying water lost to evaporation.
After 5 and 10 days 2 and 4 replicates respectively were sampled for survival and growth. Endpoints measured were growth rate and mortality after 10 days exposure.
TBBPA-DBPE was not detected in the *C. dilutes*. (However the limit of detection was not given in the study.)

Results:
The assay did not fulfil the validity criteria of 80% survival in the control as the negative control had a survival of 73% and the solvent control a survival of only 27%. Validity criteria: emergence in the controls must be at least 70%.
The weight of larvae were decreased dose-response dependent, with a statistically significant decrease at 9 000 ng/g weight sediment (see results below). The standard

deviation was rather high in the concentrations up to 900 ng/g ww sediment, but small at the treatment 9 000 ng/g ww.
The test is assessed with Klimisch 3 because of the high mortality values in the controls.

Table 10

CHIRONOMUS WEIGHT AND MORTALITY (VALUES READ FROM GRAPH)		
Concentration ng/g wet weight sediment	**Mortality %**	**Weight (g)**
Solvent control	73	0.0089
Negative control	27	0.0171
90	75	0.0046
900	48	0.0037
9000	73	0.0016

***Chironomus riparius* -Toxicity** (TL, 2005a)

A test with *Chironomus riparius* was conducted according to OECD TG 218 (Sediment-water chironomid toxicity test using spiked sediment) with TBBPA-DBPE. Two test items were used (test item A and test item B). Test item A (common name PE-68) with a purity of 99% was not radio labelled, test item B was ^{14}C radio labelled in the aromatic ring (purity > 99%). The ratio of test item A to test item B in the test vessels was not given in the description of the preparation of test vessels. For analytical measurement the radioactivity was measured by Liquid scintillation counting in the sediment (after combustion) and in the water phase (without combustion). The animals were exposed over spiked sediment and the animals received unspiked food. According to OECD TG 218 it is recommended to use spiked food for substances with logKow > 5 *("For strongly adsorbing substances (e.g. with log Kow > 5) or for substances covalently binding to sediment, ingestion of contaminated food may be a significant exposure route. In order not to underestimate the toxicity of highly lipophilic substances, the use of food added to the sediment before application of the test substance may be considered."*). Feeding with unspiked food can possibly reduce exposure via sediment ingestion (ECHA guidance R.10.5.2.2). Since the chironomids received unspiked food the test is less reliable.
The test duration was 28 d, an additional test was conducted to evaluate survival and growth with a duration of 10 d. First instar larvae with an age of about 10 d were placed in a defined artificial sediment substrate spiked with the test item in the concentrations: 10, 30, 90, 270, 810 mg/kg dry sediment with three replicates per treatment and control. Per replicate 20 larvae were introduced. For the preparation of the test vessels acetone was mixed with the test solution and then with a portion of sand. The acetone was evaporated and then the sand test item mixture was mixed with the artificial sediment. The same amount of acetone/sand (acetone evaporated) was also added to the control. Glass plates covered the vessels.
Analytical results: The measured concentrations of the test substance were in the range of 74.5 to 117.2 % of nominal in the sediment. However as the most concentrations were in the range of 80 to 100 % of nominal and as no effects were seen, the nominal concentrations were used.

Results:
No effects on survival, growth, development rate and emergence were seen. The NOEC is >= 810 mg/kg dry sediment.
The test is not used for PNEC derivation since the chironomids received unspiked food (explanation see above).

Table 11

SEDIMENT ORGANISMS TOXICITY TESTS					
Species	**Substance tested**	**Duration / Concentration / test condition**	**Result**	**Comments**	**Reference**
Hyalella azteca	Technical TBBPA-DBPE (FR-720)	70 d In sediment: 0.00235 ng/g OC (mean of two ponds, very low deviation); Concentration in particulative phase: 0.454 and 0.556 ng/g OC (two ponds = two replicates)	No effects on mortality, biomass. In one pond no reproduction, in the other pond high reproduction	Mesocosm study, study 2009	(de Jourdan, 2012)
Hyalella azteca	TBBPA-DBPE Purity 99% (test item A, PE-68) and >99% (test item B, radio-labeled)	Guideline OPPTS 850.1735 (draft 1996) 28 d Conc: 22.7, 71.6, 209, 642, 1837 mg/kg (m); 8 replicates Conditions: defined artificial sediment and flow-through, Age of organisms: 7 to 8 days	NOEC length: 642 mg/kg dry sediment No effects on survival and weight.	Validity criteria were met, control survival above 80%.	(TL, 2005b)
Chironomus dilutus	Technical TBBPA-DBPE (FR-720)	According to OECD TG 218 (with deviations), Duration only 10 d 90, 900, 9000ng/g wet weight sediment, age of larvae 10 – 12 days after hatching, 6 replicates per treatment, 10 larvae per replicate; static, solvents: iso-propanol and toluene (ratio 50:1)	Significant decreased weight at 9000ng/g ww compared with both controls. (dose dependency existed) Effects on mortality cannot be determined (high control mortality)	High control mortality (validity criteria not fulfilled), TBBPA-DBPE was not detected in the C. dilutes.	(de Jourdan, 2012)
Chironomus riparius	TBBPA-DBPE Purity 99% (test item A, PE-68) and >99% (test item B, radio-labeled)	OECD TG 218, duration 28 d; additional test for survival and growth: 10 d; defined artificial sediment substrate spiked with the test item; Age of larvae: about 10 d; Concentrations: 0, 10, 30, 90, 270, 810 mg/kg dry sediment; 3 replicates per treatment and control	No effects on emergence and development rates after 28 d. No effects on survival and growth after 10 d up to 810 mg/kg	Validity criteria are fulfilled: control survival 90%, chironomids received unspiked food (less reliable)	(TL, 2005a)

7.8.1.5. Other aquatic organisms

No further data is available.

7.8.2. Aquatic compartment (including sediment) for TBBPA-DBMPE

7.8.2.1. Fish

For TBBPA-DBMPE no studies on fish are available.

7.8.2.2. Invertebrates

A test according OECD TG 202 with TBBPA-DBMPE is available in the registration dossier (TL, 2015) and on the ECHA dissemination site. Daphnia magna were tested in semi-static limit test with the concentration 10 µg/L (nominal). The measured concentration were in the range of 81 % and 109 % of nominal. Acetone in a concentration of 0.1 mL/L was used as solvent.

The test lasted 48 h. No effects were observed. Therefore the EC50 is > 10 µg/L.

A long term toxicity test for invertebrates is not available.

7.8.2.3. Algae

For TBBPA-DBMPE no studies on algae are available.

7.8.2.4. Sediment organisms

For TBBPA-DBMPE no studies on sediment organisms are available.

7.8.3. Terrestrial compartment for TBBPA-DBPE

Earthworm acute toxicity (prolonged for reproduction), (TL, 2004)

A test with *Eisenia fetida* was conducted in 2004 with TBBPA-DBPE. The test was conducted according OECD TG 207, Earthworm acute toxicity, however the duration was prolonged and a reproduction test performed. Effect on biomass and mortality of the adult worms were determined after 28 d. After 56 d effects on reproduction were determined by counting the offspring. This procedure is similar to the OECD TG 222 (Earthworm Reproduction Test). The age of worms was between 2 and 3 months, the wet mass of worms between 300 and 600 mg.

Test item A (purity 99% TBBPA-DBPE, common name PE-68) and B (purity > 99% TBBPA-DBPE) were used. Test item B was ^{14}C-labelled in the aromatic ring.

The test substrate was an artificial soil that contained TBBPA-DBPE in the different test concentrations. Concentrations used were: 8, 16, 32, 64, 128, 256, 512 and 1024 mg/kg dry mass (nominal) with four replicates per concentration and control.

Analytical methods: The radioactivity in the artificial soil was determined by combustion and the evolved $^{14}CO_2$ was quantified. These results were linked to the test item concentration (amount) by comparison to the nominal radioactivity of the mixed test items A (non labelled) and B (labelled). The measured concentrations were in the range of 75 to 101.4 % of nominal values. The nominal values were used for calculating the effect values (at test start measured concentrations were 87 to 101%, after 56 d one concentration was measured to be 75% of nominal but not in the range where an effect was seen, 79.3% was measured at the nominal concentration of 1024mg/kg)

Results:
An effect on reproduction was seen after 56 d. The number of offspring decreased by 18% at 1024 mg/kg dry mass was assessed to be the LOEC. The NOEC is 512 mg/kg dry mass ($p < 0.05$; calculated with *Williams Multiple t-test* for homogeneous variances). No effects appeared on mortality and weight change (biomass) after 28 days.

Table 12

TERRESTRIAL INVERTEBRATES TOXICITY TESTS					
Species	**Substance tested**	**Duration / Concentration / test condition**	**Result**	**Comments**	**Reference**
Eisenia fetida	TBBPA-DBPE Purity 99% (test item A, PE-68) and >99% (test item B, radio-labelled)	According to OECD TG 207 (Earthworm acute toxicity), prolonged for reproduction similar to OECD TG 222. Duration: 56 d for reproduction, 28 d for mortality and weight; 8, 16, 32, 64, 128, 512, 1024 mg/kg dry mass (n), measured concentrations were at day 0: 87-101%, after 56 d: 75-97%; 4 replicates for treatments and control	NOEC 512 mg/kg soil dw (reproduction, nominal) LOEC 1024 mg/kg soil dw (reproduction) NOEC: >= 1024 mg/kg soil dw (mortality and biomass) LOEC > 1024 mg/kg soil dw (mortality and biomass)	Validity criteria fulfilled	(TL, 2004)

7.8.4. Terrestrial compartment for TBBPA-DBMPE

For TBBPA-DBMPE no studies for the terrestrial compartment are available.

7.8.5. Microbiological activity in sewage treatment systems

7.8.6. PNEC derivation for TBBPA-DBPE and other hazard conclusions

Table 13

PNEC DERIVATION AND OTHER HAZARD CONCLUSIONS		
Hazard assessment conclusion for the environment compartment	**Hazard conclusion**	**Remarks/Justification**
Freshwater	n.a.	No effects were seen.
Marine water	n.a.	No data
Intermittent releases to water	n.a.	n.a.
Sediments (freshwater)	PNEC: 6.42 mg/kg	Assessment factor: 100
Sediments (marine water)	PNEC: 0.642 mg/kg	Assessment factor: 1000
Sewage treatment plant	n.a.	n.a.
Soil	PNEC: 5.12 mg/kg	Assessment factor: 100
Air	n.a.	n.a.
Secondary poisoning	n.a.	n.a.

7.8.7. Conclusions for classification and labelling for both Substances

N/A

7.9. Human Health hazard assessment for both Substances

Not assessed in this substance evaluation.

7.10. Assessment of endocrine disrupting (ED) properties for both Substances

7.10.1. Endocrine disruption – Environment

TBBPA-DBPE and TBBPA-DBMPE were evaluated jointly as they are similar compounds where the only difference is an additional methyl group on each of the two alkyl chains of TBBPA-DBMPE in comparison to TBBPA-DBPE (see section 7.14). QSAR data are depicted for both substances and compared. In vitro data are only available for TBBPA-DBPE, and reference is made to TBBPA-DBMPE.

For some biotransformation products of TBBPA-DBPE and TBBPA-DBMPE, QSAR data are available that will be used to show indication for endocrine activity. However, these biotransformation products can only be formed under very special anaerobic conditions, see section 7.10.1.2.2. As it is possible that these substances are formed in organisms they are of relevance for the assessment and are included in the following sections.

7.10.1.1. Qualitative Structure/Activity Relationship (QSAR)

QSAR data regarding ED

Due to structural similarity between TBBPA-DBPE and TBBPA-DBMPE the properties are regarded to be conferrable between the two substances. QSAR data are only used as supporting information. The data indicate that also the potential metabolism product of TBBPA-DBPE : TBBPA-MDBPE (TBBPA mono(2,3-dibromopropyl ether)) and the potential metabolism product of TBBPA-DBMPE: TBBPA-MDBMPE (TBBPA mono(2,3-dibromo methyl ether)) are of concern for endocrine activity for the thyroidal and antiandrogen pathway. These metabolites are an intermediate structure between TBBPA-DBPE or TBBPA-DBMPE TBBPA on the one side (both hydroxyl groups capped by an ether function) and TBBPA (with two free hydroxyl groups) and are therefore relevant for ED assessment. They are formed during biotransformation under very special conditions. The QSAR considerations are also seen in connection to QSAR for TBBPA. The substances above have both properties of the source substances and TBBPA and are because of potential endocrine activity relevant for the assessment.

Thyroid mode of action

QSAR data from the Danish QSAR database (http://qsardb.food.dtu.dk/db/index.html): The Danish QSAR database contains predicted data for thyroid receptor α and ß binding from the databases CASE Ultra, Leadscope and SciQSAR. The QSAR results show indication for thyroid Mode of action for TBBPA-DBPE and TBBPA-DBMPE, but at high concentrations (at least an IC_{50} of 17.5 mg/L for TBBPA-DBPE and 12.8 mg/L for TBBPA-DBMPE in the database Leadscope Enterprise for human Thyroid hormone Receptor ß (hTRß) binding).

Further QSAR predictions were made regarding Thyroperoxidase Inhibition by the Danish CA using a second QSAR model (QSAR2) (Rosenberg 2017). Both substances (TBBPA-DBPE and TBBPA-DBMPE (similar substance, read-across substance) come out with inconclusive results. The probabilities are 0.401 for TBBPA-DBPE and 0.385 for TBBPA-DBMPE. The probability is in the grey area between positive and negative, since the cutoff for positive is set at 0.5 and the cut-off for negative is set at 0.3. This means that alerts for TPO inhibition are found in the substances, but not enough to raise the probability of a positive result above the cut-off of 0.5.

However, both results may give indications for an thyroid mode of action.

For the potential unsymmetric biotransformation metabolites[6] TBBPA-MDBPE (TBBPA mono(2,3-dibromopropyl ether)), TBBPA-MDBMPE (TBBPA mono(2,3-dibromopropyl methyl ether) and TBBPA-MAE (TBBPA mono(allyl ether)) no data are available. But conclusions can be drawn based on structurally similar substances:

TBBPA-MDBPE and TBBPA-MDBMPE can be assessed by read-across from TBBPA-DB(M)PE and TBBPA since TBBPA-MDBPE has a brominated alkylchain only on one ring and on the other ring is a single hydroxyl group like in TBBPA. The QSAR data that are available for TBBPA-DB(M)PE and TBBPA indicate that the two unsymmetric metabolites may have a thyroid mode of action.

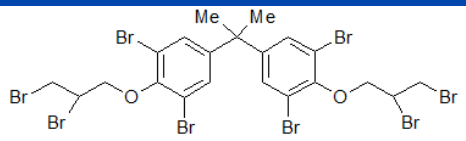
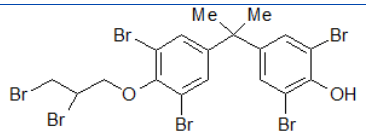
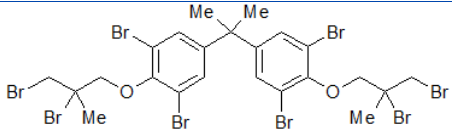
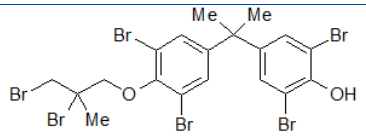
The other potential biotransformation product TBBPA-MAE (TBBPA mono(allyl ether), see (Liu et al., 2017)) can be assessed by read-across from TBBPA and the substance TBBPA bis(allyl ether) (TBBPA-BAE) since TBBPA-MAE consists of an allyl ether chain on one ring, and on the other ring is the hydroxyl group like in TBBPA. For TBBPA-BAE, indication for thyroid receptor α and ß binding was seen (stronger binding was predicted for TR ß binding (IC50 between 26 and 233 mg/L) than for TR α binding (IC_{50} between 369 and 1511 mg/L).

For the purpose of this read-across the data regarding TR binding for TBBPA are included: TR α binding: IC50 between 58 and 86996, TR ß binding: IC50 between 10 and 17599 mg/L.

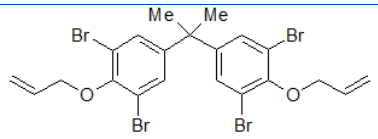
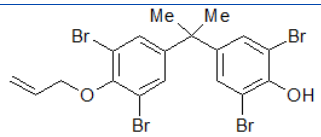
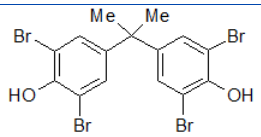
In all cases where data are available it can be seen that binding to TR ß has a lower IC50 value than binding to TR α. The data for TBBPA-DBPE, TBBPA-DBMPE, TBBPA-DPMPE and TBBPA-BAE and TBBPA are in the same range and the lowest values were always in the database Leadscope (IC_{50}: 10 to 26 mg/L).

In summary, it is reasonable that effects may occur via perturbation of the thyroid pathway.

Table 14

QSAR predictions using the Danish QSAR database: Thyroid receptor binding					
Name	**Structure**	**TR α binding IC50 mg/L**	**Inside applicability domain in the three databases**	**TR ß binding IC50 mg/L**	**Inside applicability domain in the three databases**
TBBPA-DBPE		236.8 to 9497	Yes: 2 of 3	17.5 to 603.9	Yes: 2 of 3
TBBPA-MDBPE		No values available, but structure between TBBPA-DBPE and TBBPA.			
TBBPA-DBMPE		196.8 to 7535	Yes: 2 of 3	12.8 to 1111	Yes: 2 of 3
TBBPA-MDBMPE		No values available, but structure between TBBPA-DBMPE and TBBPA.			

[6] The transformation product may be formed only in organisms under anaerobic conditions (see section 7.10.1.2.2 and there the subparagraph 'Metabolism under anaerobic conditions').

TBBPA-BAE		369 to 1511	Yes: 2 of 3	26.1 to 233	Yes: 2 of 3
TBBPA-MAE		No values available, but structure between TBBPA-BAE and TBBPA.			
TBBPA[7]		58.1 to 86996	Yes: 3 of 3	10 to 17599	Yes: 3 of 3

Androgen receptor antagonism

The Danish QSAR database gives indications for an antiandrogen mode of action for TBBPA-DBPE and TBBPA-DBMPE. For TBBPA-DBPE, two of three results predict antiandrogenicity and are inside the applicability domain. For TBBPA-DBMPE, two of three results predict antiandrogenicity too, but one of them is outside the applicability domain. Further details see in the table below.

For TBBPA-BAE, two of three results predict antiandrogenicity and are inside the applicability domain. For TBBPA-MAE (TBBPA mono(allyl ether), a potential metabolism product of TBBPA-DBPE) no data are available.

For TBBPA-MDBPE, potential metabolism product of TBBPA-DBPE) no data are available, however in the structure it is between TBBPA DBPE and TBBPA.

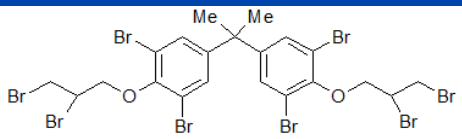
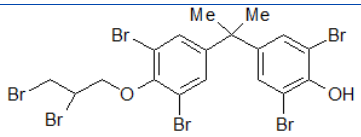
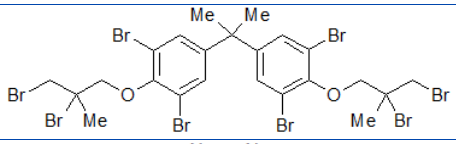
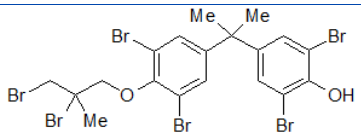
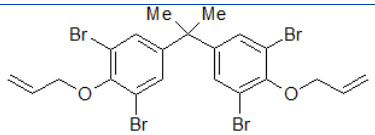
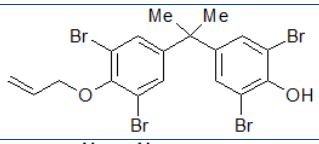
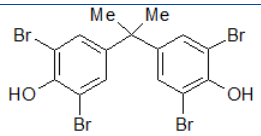
For TBBPA-MDBMPE, a potential metabolism product of TBBPA-DBMPE) no data are available, however in the structure is between TBBPA DBMPE and TBBPA.

Data for TBBPA predict antiandrogenicity in three out of three cases and are inside the applicability domain. The Danish QSAR database gives for the androgen receptor antagonism only structural alerts and no estimation of effect values.

It can be assumed that also the metabolism products TBBPA-MDBPE, TBBPA-MDBMPE and TBBPA-MAE show similar QSAR predictions as the other substances in the table below, because the structures correlate partially with the substances that have two alkyl chains (on each ring one chain) and with TBBPA that has no alkyl chain and the metabolites have on one ring the same phenolic brominated ring structure like TBBPA.

[7] Only values are included that are inside the applicability domain. TBBPA is inside the applicability domain of the database CASE ultra, where the other substances are always outside the applicability domain. The values for TBBPA in this database are extremely high. Therefore the range of TBBPA is very high.

Table 65

QSAR predictions using the Danish QSAR database: Androgen receptor antagonism					
Name	**Structure**	**Androgen receptor antagonism Applicability domain: inside**		**Androgen receptor antagonism Applicability domain: outside**	
		Anti AR positive	**Anti AR negative**	**Anti AR positive**	**Anti AR negative or inconclusive**
TBBPA-DBPE		Positive 2 of 3			Negative 1 of 3
TBBPA-MDBPE		No values available, but structure between TBBPA-DBPE and TBBPA			
TBBPA-DBMPE		Positive 1 of 3		Positive 1 of 3	Inconclusive 1 of 3
TBBPA-MDBMPE TBBPA mono(2,3-dibromomethyl ether)		No values available, but structure between TBBPA-DBMPE and TBBPA			
TBBPA-BAE		Positive 2 of 3	Negative 1 of 3		
TBBPA-MAE		No values available, but structure between TBBPA bis(allyl ether) and TBBPA			
TBBPA Tetrabromo-bisphenol A		Positive 3 of 3			

Estrogen receptor

The Danish QSAR database shows one positive result for estrogen receptor α binding (Leadscope), but otherwise 7 negative or inconclusive results. The QSAR Toolbox did not show a binding potential to the estrogen receptor. The UBA (Umweltbundesamt) ED (Endocrine disruption) screening tool did not show structural alerts for the ER binding for TBBPA-DBPE and TBBPA-DBMPE.

7.10.1.2. Endocrine *in vitro* tests

The information on endocrine activity of TBBPA-DBMPE was evaluated using information from *in vitro* tests of the read-across substance TBBPA-DBPE.

There is information available on 15 *in vitro* tests regarding TBBPA-DBPE. Three tests are of minor importance for the substance evaluation because the connection to an endocrine mode of action is unclear. The increased aryl hydrocarbon receptor (Ahr)-mediated transcriptional activity and the up regulation of CYP1a4 in chicken embryo hepatocytes (Ma et al. 2015) can probably show that TBBPA-DBPE is going to be metabolized, however a relevant MoA for ED assessment is not covered by these assays. In a dioxin receptor (AhR mediated arylhydrocarbon receptor) calux assay no effects appeared (Hamers et al. 2006). These tests are not further described below but included in

Table 16 for completeness.

Overview on the most important in vitro effects of TBBPA-DBPE:

Aromatase CYP 19

Canton et al. 2005 (Canton et al., 2005) exposed cells (H295R human adrenocortical carcinoma cell line) to TBBPA-DBPE (or TBBPA or the polybrominated diphenyl ethers BDE 206 and BDE 209 and other BDEs) at concentrations from 0.5µM to 7.5µM in order to evaluate the effect on aromatase (CYP 19) induction or inhibition of their catalytic activity. The exposure duration was 24 h.

TBBPA-DBPE caused at 7.5 µM (7.08mg/L) an inhibition of the enzymes catalytic activity of 41.1% ±19.4% ($p<0.05$) compared to the untreated control. The positive control (4-Hydroxyandrostenedione, 4-HA, 10 µM) caused an inhibition of 90% of the maximum aromatase activity of the untreated control.

In contrast to TBBPA-DBPE the decrease caused by TBBPA (in blue, for comparison see

Table) was marginal. The results for TBBPA-DBPE and the polybrominated diphenyl ethers BDE 206 and BDE 209 (in grey) are rather similar as can be seen in the

Table . The polybrominated diphenyl ethers BDE 206 and BDE 209 are also highly brominated and have a high log Pow and are very poorly soluble in water. They have two aromatic rings, however with a different kind of binding (ether binding) between the rings.

CYP 19 is an enzyme that mediates the conversion of androgens to estrogens during aromatization. By inhibition of that enzyme less estradiol will be produced and hence it can be considered an anti-estrogenic effect.

CYP 17

Canton et al. 2006 (Canton et al., 2006) evaluated also effects on the enzyme CYP 17 using the H295R human adrenocortical carcinoma cell line by exposure to 0.01 to 10µM of TBBPA-DBPE or TBBPA. CYP 17 was not inhibited by both substances. CYP 17 is an enzyme that also plays a key role in the steroidogenic pathway by synthesizing dehydroepiandrosterone (DHEA) and androstenedione.

Sulfotransferase (SULT1E1)

Inhibition of sulfotransferase (SULT1E1) enzymes was seen in the publication by Hamers et al. 2006 (Hamers et al., 2006). The recombinant human sulfotransferase 1E1 (SULT1E1) was expressed in a V79-1E1 Chinese hamster cell line. Hamers et al. used for their assessment the technical product of TBBPA-DBPE, TBBPA or further brominated flame retardants (BFRs). Regarding the technical product of TBBPA-DBPE the author stated *"…the TMs of HBCD and TBBPA-DBPE may still be contaminated with other intermediate compounds. In particular, a possible contamination of TBBPA-DBPE with TBBPA could explain the (very) high potency of TBBPA-DBPE in the TTR and E2SULT assays."*

> The maximum tested concentration of TBBPA-DBPE was 10µM. The reference substance (positive control) was Pentachlorophenol (PCP) at 10µM. DMSO (1%) was used as solvent. Sulfotransferase was inhibited by the technical product of TBBPA-DBPE with an IC50 of 0.27 ± 0.11µM and TBBPA (in blue, in

Table below) inhibited with an IC50 of 0.016 ± 0.007µM (IC50 = half maximal inhibitory concentration). It was proposed by Kester et al. (2002) that the inhibition of sulfotransferase enzymes leads to a decreased sulfation of estradiol (E2) and thereby to an increased bioavailability of estrogens. Hence it would be an estrogenic mode of action.

Hamers et al. 2008 (Hamers et al., 2008) provided a publication where the same tests were conducted as in the publication of Hamers et al. 2006, but a metabolical activation was performed with all substances before testing. No information regarding sulfotransferase inhibition by TBBPA-DBPE was provided in this publication.

AR CALUX assay

In the publication by Hamers et al. 2006 no effect appeared in the AR CALUX assay. However in the publication of Hamers et al. 2008 antiandrogenic activity of the technical product of TBBPA-DBPE in the AR Calux assay was shown after biotransformation (metabolical activation). The biotransformation was conducted using rat hepatic microsomes from phenobarbital exposed male rats. The CALUX (chemically activated luciferase gene expression) bioassays examines the potency to interact with the respective receptor, in this case with the androgen receptor (AR).

The anti AR CALUX assay (AR inactivation in the DHT (dihydrotestosterone)-activated AR-CALUX bioassay) was conducted using U-2 OS cells (human osteoblast cells) in the presence of DHT (164 pM) to evaluate the antiandrogen effect. As positive control flutamide was used (IC50=1300nM). The concentrations of TBBPA-DBPE used were 100 and 1000 nM and a control without TBBPA-DBPE. At 100 nM (94.4 µg/L) the activity was 74% compared to 100% of the negative control, at 1000 nM (944 µg/L) the activity was 63% (these values were read from a graph). That means an AR inactivation at 94.4µg/L of 26% and at 944µg/L of 37%. There was a high standard deviation at 94.4µg/L, but a very low SD at 944µg/L. A dose-response existed. The significance was not calculated.

TTR-binding assay

Hamers et al. (2006) also examined the thyroxine (T4) replacement by TBBPA-DBPE (technical product), TBBPA or different BFRs from the human transthyretin (TTR), the T4-transporting protein in plasma. Thyroxine is the precursor of the active thyroid hormone 3,3',5-triiodothyronine (T3). The maximum tested concentration of TBBPA-DBPE (techn. product) or TBBPA was 62.5 µM. The treatments contained 2.5% DMSO. Radioactive-labeled (^{125} I-labeled thyroxine) and unlabeled T4 were used in a mixture. After reaching binding equilibrium (incubated overnight) the radioactivity of the TTR-bound ^{125} I-T4 – containing eluate was measured and corrected for the initial amount of ^{125} I-T4 added before incubation started. The test was conducted according to Lans et al. 1993 (Lans et al., 1993) with modifications.

TBBPA-DBPE (technical product) had an IC50 of 5.2 µM; TBBPA had a stronger activity with an IC50 of 0.031 µM.

Hamers et al. (2008) conducted the same TTR-binding assay with the technical product of TBBPA-DBPE also after biotransformation. No activity was then seen up to 10 µM (IC50 > 10 µM). Also TBBPA has a lower activity after biotransformation (IC50 of 0.1 – to 1 µM after biotransformation).

T-Screen

Hamers et al. (2006) assessed also the substances TBBPA-DBPE (technical product), TBBPA or different BFRs with the T-screen using rat pituitary tumor cell line (GH3 cells) to examine the T3-dependent cell proliferation. The concentrations tested were 0.001–1000nM in the absence or presence of 0.25µM T3. All treatments contained 0.5% DMSO. After 96h of exposure the cell proliferation was determined.

TBBPA-DBPE (technical product) had no effect on cell proliferation. TBBPA had an effect at 0.5µM (carried out with additional 0.25nM T3) that enhanced the cell proliferation by 23% compared with the maximum T3-induced response. Without additional T3 no effect appeared by TBBPA too.

***In vitro* tests without effects**

There were other tests examined in the publications by Hamers et al. 2006 and Hamers et al. 2008, which showed no effects by TBBPA-DBPE (technical product) and TBBPA. The

tests show, that both substances do not elicit estrogen agonistic or antagonistic effects that are mediated by ER (up to 12.5µM), they are not androgen or progesterone active (up to 10µM).

Likewise, no estrogen and anti-estrogen effect, no androgen and anti-androgen effect were observed by Ezechias etal 2012 (Ezechias et al., 2012), who conducted two yeast reporter-gene assays (ß-Galactosidase assay and bioluminescent screen) to examine the potential of substances to interfere with estrogen and androgen pathways. Also no toxic effect on cells were observed. However, the tests were conducted without metabolical activation and therefore there is no discrepancy regarding the above described anti androgenic effect. Further in vitro evaluations by different authors (Liu et al., 2016c; Ma et al., 2015; Wielogorska et al., 2015) are listed in the table below, which are negative and therefore not further elucidated here. They show, that TBBPA-DBPE does not up regulate VTG-mRNA in chicken embryo hepatocytes up to 300µM (Ma et al. 2015) and that cell viability was not affected up to 300µM. Further they show that TBBPA-DBPE was not estrogenic or antiestrogenic in a reporter gene assay where luciferase activity was measured (Wielogorska et al., 2015). Hamers et al. (2006) and Hamers et al. (2008) showed that in an ER Calux assay using human breast cancer cells no effect on estrogen receptor appeared with and without metabolical activation (up to 12.5µM). Also from Hamers et al. (2006) and (2008) a PR Calux assay using U-2 OS (human osteoblast cells) showed no effect on the progesterone receptor (PR) with and without metabolical activation up to 10µM of the technical product of TBBPA-DBPE. (Liu et al., 2016c) showed that TBBPA-DBPE was not neurotoxic up to 100µM using PC12 cells (from rat adrenal tumors, cells have similar biological characters with neural cells and are widely used in various neurological studies).

Table 6 summarises all *in vitro* effects. Effects on other BFRs are included for comparison in smaller lettering: TBBPA (in blue) because of being a potential degradation product of TBBPA-DBPE; BDE 206 and 209 (in grey, bromodiphenyl ether) as brominated BFRs that are also very poorly soluble in water. BDE 206 has 9 bromine atoms and BDE 209 has 10 bromine atoms in the molecule. In comparison TBBPA-DBPE has 8 bromine atoms. Remarkably, only BDE 206 and BDE 209, which are highly brominated biphenyl ethers did inhibit CYP19 aromatase activity similar to TBBPA-DBPE, whereas the lower brominated biphenyl ethers (with 7 and less bromine atoms) did not inhibit aromatase.

Table 16 For comparative purposes, this Table also comprises coloured entries which are given for structurally similar substances (TBBPA: blue) or other brominated aromatic flame retardants (bromodiphenylether (BDE) derivatives: grey) alongside data on TBBPA-DB(M)PE.

In vitro tests						
Substance	**Cell type**	**Kind of effect, test system**	**Concentration/vehicle**	**Effect**	**Reference**	**Comment**
TBBPA-DBPE (from Broom-chemie, Terneuzen, The Netherlands), purity unknown	H295R human adrenocortical carcinoma cell line	**Inhibition of aromatase (CYP 19) activity,** exposure 24 h	0.5µM to 7.5µM Vehicle: DMSO (0.1% v/v)	At 7.5µM (7.08 mg/L) significant decreased aromatase activity by 41 %	(Canton et al., 2005)	**Anti**estrogenic effect at 7.5µM: 58.9% (+-19.4%) remaining activity compared to untreated control (100%) Positive control for aromatase inhibition: (4-Hydroxy-androstenedione (4-HA, 10 µM)), remaining aromatase activity: 10% of untreated control
BDE 206 Nonabromodiphenyl ether	H295R human adrenocortical carcinoma cell line	Inhibition of aromatase (CYP 19) activity, exposure 24 h	See above	At 7.5µM decreased aromatase activity by 39 %	Canton et al. 2005	Remaining activity: 61.3 % +- 5.5 of untreated control
BDE 209 Decabromodiphenyl ether	H295R human adrenocortical carcinoma cell line	Inhibition of aromatase (CYP 19) activity, exposure 24 h	See above	At 7.5µM decreased aromatase activity by 36 %	Canton et al. 2005	Remaining activity: 63.7% +- 5.9 of untreated control
TBBPA (Purity > 99%)	H295R human adrenocortical carcinoma cell line	Inhibition of aromatase (CYP 19) activity	See above	No significant effect	Canton et al. 2005	No effect

TBBPA-DBPE (TM) Broomchemie (Terneuzen, NL).	H295R human adrenocortical carcinoma cell line	Inhibition of CYP 17 Solvent: DMSO, duplicate experiments, each conc. in quadruplicate, exposure: 24 h	0.01 to 10µM Vehicle: DMSO	No effect	(Canton et al., 2006)	No effect
TBBPA-DBPE (technical product)	Recombinant human sulfotransferase 1E1 (SULT1E1) expressed in a V79-1E1 Chinese hamster cell line	**Inhibition of estradiol sulfotransferase** (SULT1E1) enzymes	Maximum concentration 10µM DMSO (1%)	IC50 0.27 ± 0.11µM	(Hamers et al., 2006)	Estrogenic effect (decreased sulfatation and hence decreased clearance of estradiol) Positive control: Pentachlorophenol (PCP): IC50 0.15 ± 0.06 µM
TBBPA	See above	Inhibition of estradiol sulfotransferase (SULT1E1) enzymes	DMSO (1%)	IC50: 0.016µM +- 0.007	Hamers et al., 2006	Estrogenic effect
TBBPA-DBPE (technical product)	Human TTR (prealbumin from human plasma)	**T4 replacement**	DMSO (2.5%)	IC50: 5.2µM After Biotransformation with rat liver microsomes: IC 50 > 10µM	(Hamers et al., 2006) (Hamers et al., 2008) (Biotransformation)	Thyroidal without biotransformation Not thyroidal after biotransformation up to 10µM Similar values like BDE-181 Positive control: T4: IC 50 0.055µM
TBBPA	Human TTR (prealbumin from human plasma)	T4 replacement	DMSO (2.5%)	IC_{50} 0.031µM After Biotransformation with rat liver microsomes: IC 50 0.1 to 1µM	Hamers et al., 2006 Hamers et al., 2008 (Biotransformation)	Thyroidal, decreased activity after biotransformation
TBBPA-DBPE (technical product)	rat pituitary tumor cell line (GH3 cells)	T-Screen, exposure 96 h	Concentration 0.001 nM to 1µM, DMSO 0.5%	No effect	(Hamers et al., 2006)	no thyroidal effect

TBBPA-DBPE (technical product)	U-2 OS (human osteoblast cells)	**AR-CALUX assay** in the presence of DHT (164 pM) With biotransformation (only Hamers 2008)	Maximum conc: 10µM DMSO (0.2v/v)	AR-antagonistic after biotransformation (at 100nM (94.4µg/L): 74%, at 1000nM (944µg/L): 63% of positive control Without biotransformation no AR- effect.	(Hamers et al., 2008) (with biotransformation) (Hamers et al., 2006) (without biotransformation)	With biotransformation: AR-antagonistic effect observed (without statistics) Without biotransformation: not AR antagonistic. Positive control: flutamide
TBBPA-DBPE (technical product)	U-2 OS (human osteoblast cells)	PR-CALUX assay	Maximum conc: 10µM DMSO (0.2v/v)	Without and with biotransformation no PR-effect.	(Hamers et al., 2008) (with biotransformation) (Hamers et al., 2006) (without biotransformation)	No effect on progesterone receptor
TBBPA-DBPE (technical product)	T47D (human breast cancer cells)	ER-CALUX assay with (2008) and without (2006) biotransformation	Maximum conc: 12.5µM, DMSO (0.5v/v)	No effect	(Hamers et al., 2006), (Hamers et al., 2008)	Not ER agonistic or antagonistic with and without biotransformation
TBBPA-DBPE	Yeast strain: S. cerevisiae For androgen/ anti-androgen assay: bioluminescent yeast strain	Estrogen: Recombinant yeast assay; Anti-estrogenic assay in response to 3.7E-9M E2. Anti-androgen assay in response to 29E-9M Testosterone. All measurements in triplicate. DMSO 3%.	Maximum conc: 2.1µM	No effect	(Ezechias et al., 2012)	No estrogen and antiestrogen effect, no androgen and antiandrogenic effect, no toxic effect on cells observed

TBBPA-DBPE	Chicken embryo hepatocytes	Expression of VTG mRNA Cell viability, exposure 36 h	0.01, 0.1, 1, 10, 100, 300 µM, Vehicle: DMSO	VTG mRNA not up regulated up to 300µM No effect on cell viability up to 300µM	(Ma et al., 2015)	Not estrogenic (VTG mRNA not up regulated) At 100 and 300 µM precipitation in medium, conc. uncertain. Positive control 17ß-estradiol 0.0001, 0.001, 0.01, 0.1, 1µM
TBBPA-DBPE	Human, MMV-Luc cell line	Reporter gene assay luciferase activity measured	concentration 1E-5 M	No effect	(Wielogorska et al., 2015)	Not estrogenic, not anti-estrogenic
TBBPA-DBPE (technical product) or TBBPA	H4IIE (rat hepatoma (tumor of the liver cells)	DR-Calux assay DR=dioxin receptor (AhR mediated arylhydrocarbon receptor), Reference material: TCDD	Maximum conc: 10µM DMSO (0.4v/v)	No effect	(Hamers et al., 2006)	Not DR agonistic or antagonistic (TBBPA-DBPE and TBBPA)
TBBPA-DBPE	COS-7 cells Monkey cells (like human fibroblast cells)	Luciferase reporter gene assay		Ahr1-mediated luciferase reporter gene activity significant increased at 10 and 30µM	(Ma et al., 2015)	At 10µM about 20 % and at 30µM about 28% of max. positive control (values read from graph). Positive control: 300nM TCDD
TBBPA-DBPE	Chicken embryo hepatocytes	Avian ToxChip PCR Array, mRNA expression	10 and 300 µM, Vehicle: DMSO	Cyp1a4 was up regulated at 10 and 300µM 4.9 and 5.5-fold respectively	(Ma et al., 2015)	At 300 µM substance precipitated in culture medium, concentration uncertain.
TBBPA-DBPE	PC12 cells (from rat adrenal tumors)	Cell viability, duration 2 h	Concentration 0-100 µM	No effect	(Liu et al., 2016c)	Not neurotoxic

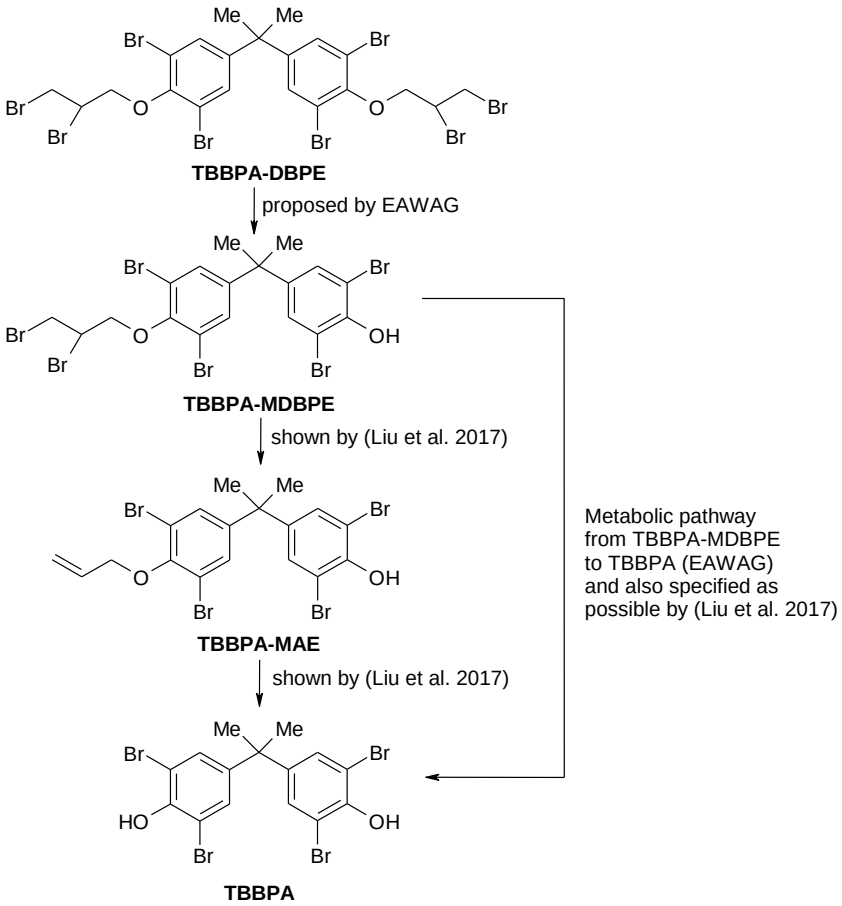
7.10.1.2.2 Biotransformation

This section on biotransformation is included, since there are several indications that endocrine active metabolits from TBBPA-DBPE and TBBPA-DBMPE might be formed. In a study fathead minnows exposed to technical TBBPA-DBPE accumulated TBBPA by day 7, which persisted in vivo over the course of the exposure (duration 41 days) and the depuration period (duration 28 days) (de Jourdan et al., 2014). The authors declared that the accumulation of TBBPA observed in the fish could be either the result of internal metabolism or direct TBBPA uptake from the environment (technical TBBPA-DBPE possibly contains TBBPA as significant impurity) or both. However, the authors noted that fathead minnows have the metabolic capability to cleave ether linkages.

Furthermore, a publication from (Liu et al., 2017) shows that an anaerobic metabolism and formation of TBBPA under special conditions from a similar substance to TBBPA-DBPE is possible (assessed more in depth below).

The possible metabolism of TBBPA-DBPE to form TBBPA or other endocrine acting substances could be of importance with respect to the endocrine activity of TBBPA-DBPE in animals. A possible metabolic pathway is depicted in Figure 1 for TBBPA-DBPE. For TBBPA-DBMPE, a similar pathway to the formation of TBBPA via the respective methylated allyl ether species can be formulated.

Figure 1: Proposed anaerobic metabolism pathway from TBBPA-DBPE to tetrabromobisphenol A (TBBPA) via tetrabromobisphenol A monoallyl ether (TBBPA-MAE).



Several observations from metabolism studies and other information regarding metabolism is available for the two substances:

Two *in vitro* metabolism tests (Knudsen et al., 2007)

For both *in vitro* metabolism studies [^{14}C] TBBPA-DBPE (phenyl-ring labelled) was used. One study was conducted with microsomal protein isolated from male rat livers, the other

with hepatocytes.
Results: No *in vitro* metabolism was observed in both kind of tests.

***In vivo* test** by (Knudsen et al., 2007) using rats (*in vivo* exposition of rats to [^{14}C] TBBPA-DBPE (phenyl-ring labelled)):

Results: The study points towards a slow hepatic metabolism of TBBPA-DBPE. Following oral exposure the majority of TBBPA-DBPE (>85 %) was eliminated in the faeces of rat by 96 h after oral exposure. The substance was poorly absorbed into the systemic circulation. Furthermore, the authors observed: "A small peak that did no co-elute with parent or known contaminant was observed. This could reflect a reaction catalyzed in the liver and/or by gut microflora."

The eMSCA sees indication for metabolisation in the in vivo data published by Knudsen et al.
In the HPLC (High pressure liquid chromatography*)* radio chromatogram (Fig. 2 D) of the faeces after oral administration of TBBPA-DBPE peaks were detected for TBBPA-DBPE, a contaminant (presumably an impurity that was also seen in the stock solution) and a further small peak named as unknown (at retention time r_T = 21.8 min), beside the peak of TBBPA-DBPE (at r_T = 23.6 min). The unknown peak was not seen in the stock solution. In blood this peak was not seen by oral or IV application (only one peak detected in blood at r_T =23.6 min), leading to the assumption that this substance could be formed in the digestive tract. For information on the presence of anaerobic bacteria in the digestive tract of vertebrates see below.

As column a C18 cartridge was used where hydrophilic substances can be seen earlier in the chromatogram than more lipophilic substances. It fits to the metabolism assumption (because substances become more hydrophilic by metabolism) that the unknown new peak is more hydrophilic because it appeared earlier on the chromatogram. As only radiolabelled substances can be seen in the radio-chromatogram it is probable, that *in vivo* metabolism of TBBPA-DBPE occurred.

***In vitro* metabolism and ED *in vitro* test**

(Hamers et al., 2008) performed *in vitro* metabolism and ED *in vitro* tests with TBBPA-DBPE. In this study no analytical identification of metabolites was done, but an endocrine test was conducted without and after metabolic activation. The *in vitro* antiandrogen CALUX (Chemical Activated Luciferares gene eXpression) assay was negative without metabolical activation, but positive after metabolical activation of TBBPA-DBPE. The biotransformation test was conducted using rat hepatic microsomes from phenobarbital exposed male rats.

Table 17

***In vitro* and *in vivo* metabolism**						
Substance	**Cell type**	**Incubation time**	**Concentration**	**Metabolism**	**Reference**	**Comment**
TBBPA-DBPE Purity 97% [^{14}C] TBBPA-DBPE	In vitro microsomal protein from male rat livers	Up to 4 h (at 10µM)	[14C] TBBPA-DBPE (10–(100)* µM, 4.8mCi/mL) was incubated with microsomal protein containing NADP+	no *in vitro* metabolism	(Knudsen et al., 2007)	*100µM was not used due to poor solubility
TBBPA-DBPE Purity 97% [^{14}C] TBBPA-DBPE	In vitro hepatocytes from male rat	Up to 4 h (at 100µM)	[14C] TBBPA-DBPE (50 and 100 µM, 190 mCi/mL) was incubated with hepatocytes	no *in vitro* metabolism	(Knudsen et al., 2007)	

TBBPA-DBPE Purity 97% [^{14}C] TBBPA-DBPE	In vivo Male Fischer-344 (F-344) rats; 8–9 weeks of age		Dosed: IV (20 mg/kg) Or oral gavage (200mCi/kg, 4 mL/kg)	In faeces (oral dosing) an unknown metabolit was detected at (r_T = 21.8 min)	(Knudsen et al., 2007)	
TBBPA-DBPE (technical product)	In vitro hepatic microsomes from phenobarbital exposed male rats microsomal protein	90 min	25 µM TBBPA-DBPE (1% v/v dilution of 2.5 mM stock solution in DMSO), 1mg/mL microsomal protein, 1mM NADPH (added to start the incubation); Control received Tris-HCl buffer instead of NADPH.	No information about metabolites, however after biotransformation TBBPA-DBPE (technical product) showed AR antagonistic activity.	(Hamers et al., 2008)	

Mesocosm study

In a mesocosm study, *P. promelas* were exposed to TBBPA-DBPE and BTBPE with an uptake period of 42 d and a depuration period of 28 d (de Jourdan, 2012). The authors concluded that *"There was metabolism of both of these compounds [TBBPA-DBPE and BTBPE (bis(tribromophenoxy)ethane) each], with both ether cleavage and debromination occurring for those compounds in fathead minnow. These metabolites persisted in vivo and had a similar fate as the parent compounds."* It was also written by Jourdan, that *"….the accumulation of TBBPA in the fathead minnow from the TBBPA-DBPE treatment could be the result of metabolism of TBBPA-DBPE or the uptake of TBBPA from the environment [from the mesocosm]. Both options are plausible as TBBPA was detected as an environmental degradation product in our 2009 study [mesocosm in 2009], and cyprinids have demonstrated the ability to cleave ether bonds (Newsome, 1995). However, the formation of TBBPA from TBBPA-DBPE in vivo has not been observed before."*

Metabolism under anaerobic conditions

These results fit to the study by Liu et al. 2017 (Liu et al., 2017) who showed that TBBPA-MDBPE (TBBPA mono(2,3-dibromopropyl ether) can be metabolised under anaerobic conditions by bacteria.

Liu et al. 2017 conducted a metabolism study using Cyanocobalamin (norpseudo-B12) under anaerobic conditions. Cyanocobalamin was identified in the anaerobic bacterium *Sulfurospirillum multivorans* by Liu et al, who showed that is formed by these bacteria.

Instead of TBBPA-DBPE, TBBPA-MDBPE was used for metabolism study since no analysis method for TBBPA-DBPE was available due to the low ionization efficiency in mass spectrometric setups.

Results of the anaerobic metabolism studies using TBBPA-MDBPE (26.9 nmol): Transformation into metabolites occurred under anaerobic conditions. After 10 minutes approx. 26% TBBPA-MDBPE and after 24 h 3% TBBPA-MDBPE was existing yet. As metabolites were formed after 10 minutes approx. 17% TBBPA-MAE and approx. 27.5% TBBPA (results were taken from graph, Figure S9. Transformation of TBBPA-MDBPE in 24 hours). Liu et al. 2017: *"The formation of TBBPA could be explained by ether bond cleavage of TBBPA-MDBPE or TBBPA-MAE."*

The eMSCA considers that also for TBBPA-DBMPE as for TBBPA-DBPE the same anaerobic metabolism pathway is relevant. Ether cleavage can be conducted in the same way as for TBBPA-DBPE.

Also in the digestive tract of vertebrates (e.g. fish) anaerobic conditions are present that enable anaerobic bacteria to live. Austin (2006), examined the bacterial microflora of fish and stated: *„A consensus view is that dense bacterial populations occur in the digestive tract (i.e., populations of up to ~108 heterotrophs g–1 and ~105 anaerobes g–1 have been reported with numbers appearing to be much higher than those of the surrounding water.*" (Austin, 2006). The presence of anaerobic bacteria in the digestive tract of rats was shown by (Li et al., 2017).

This anaerobic metabolism pathways can only occur under very special conditions: anaerobic and in presence of cyanocobalamin (Vit. B12), e.g. in organisms. The knowledge about this metabolism pathway is also applicable for TBBPA-DBPE, because the structural difference between TBBPA-DBPE and TBBPA-MBDPE is only that TBBPA-DBPE has two brominated alkyl chains and TBBPA-MBDPE has only one brominated alkylchain. Most probably TBBPA-DBPE will therefore also be metabolized like TBBPA-MDBPE via ether-bond scission between the aromatic ring and the alkylchain. As stated above this is also applicable for TBBPA-DBMPE.

Also the prediction pathway from EAWAG (http://eawag-bbd.ethz.ch/predict/) shows the pathway from TBBPA-DBMPE to TBBPA and TBBPA-MDBMPE as an intermediate step.

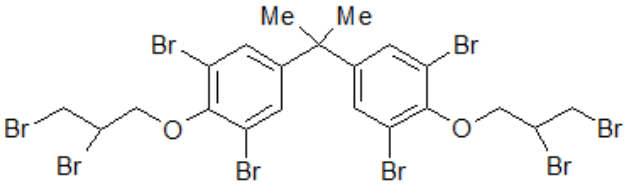
In summary, based on available information presented above the eMSCA considers that TBBPA-DBPE and TBBPA-DBMPE can be metabolized in the digestive tract of vertebrates to form endocrine active metabolites. This is e.g. indicated by the anaerobic metabolism of the similar substance TBBPA-MDBPE where the metabolism products TBBPA-MAE and TBBPA are formed under special anaerobic conditions.

For TBBPA-DBMPE the anaerobic formation of TBBPA-MAE is chemically also possible via the same mechanisms as for TBBPA-DBPE as well as the formation of TBBPA under special anaerobic conditions.

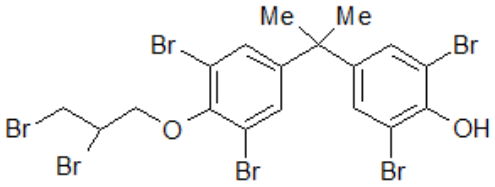
7.10.1.2.3 Structural similarity considerations between metabolites

TBBPA-MDBPE and TBBPA-MDBMPE

TBBPA-MDBPE (TBBPA mono(2,3-dibromopropyl ether)) is similar to the parent compound TBBPA-DBPE as it has one brominated alkyl chain on one ring, TBBPA-DBPE has one brominated alkyl chain on each of the two rings:

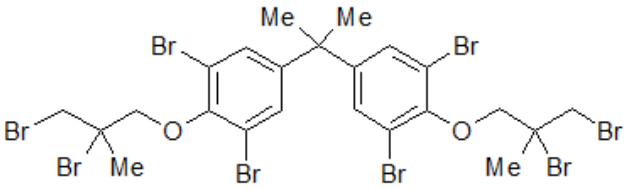


TBBPA-DBPE

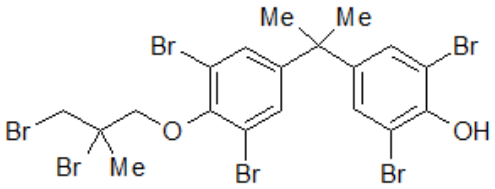


TBBPA-MDBPE

The same relation holds true for TBBPA-MDBMPE (TBBPA mono(2,3-dibromo-2-methylpropyl ether)) and its parent compound TBBPA-DBMPE:

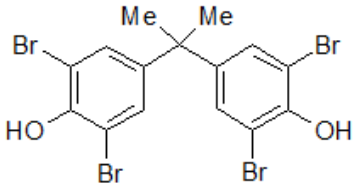


TBBPA-DBMPE



TBBPA-MDBMPE

Therefore, the two monoethers and their properties are considered to be inbetween their respective parent compounds and free TBBPA, the eventual product of complete dealkylation which bears no side chains on the hydroxyl group of the phenyl rings.



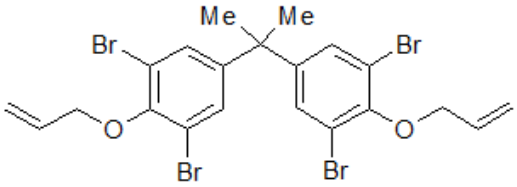
TBBPA

Postulated allyl ether metabolites for TBBPA-DBPE and TBBPA-DBMPE

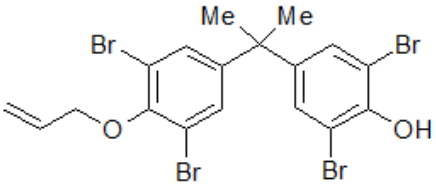
Liu et al. 2017 could show that the brominated side chain of TBBPA-MDBPE can be dehalogenated to form allyl ether species like TBBPA-MAE. In principle, the eMSCA considers that the same dehalogenation mechanism of TBBPA-DBPE (occurring on both side chains) could lead to the bisallyl ether species TBBPA-BAE.

The analogous dehalogenation reaction of TBBPA-DBMPE resp. TBBPA-MBMPE would formally lead to the bis- or monomethallyl ether species TBBPA-BMAE or TBBPA-MMAE.

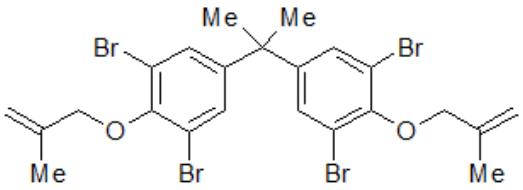
All four species are depicted below.



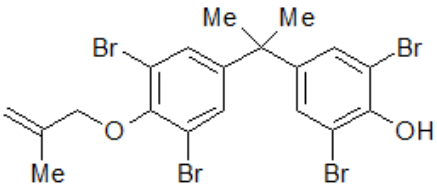
TBBPA-BAE



TBBPA-MAE



TBBPA-BMAE



TBBPA-MMAE

TBBPA-MAE is similar to the substance TBBPA-BAE (TBBPA bis(allyl ether)) but only one alkylchain exists in the molecule and the other aromatic ring has the same structure as the brominated ring system of TBBPA without alkyl chain.

For TBBPA-BAE (TBBPA bis(allyl ether)) QSAR data (antiandrogen and thyroidal, see above in the section QSAR) exist, giving indications on same properties for TBBPA-MAE.

7.10.2 Endocrine disruption - Human health for both Substances

Not assessed in this substance evaluation.

7.10.3 Conclusion on endocrine disrupting properties for the environment

The available *in vitro* tests on endocrine properties give indication for endocrine properties of the substance TBBPA-DBPE in the environment. However, a conclusion is not possible, because the in vitro tests were conducted with the technical product with unknown purity. It is possible that the effects were caused by impurities like TBBPA or other endocrine acting substances. The available aquatic in vivo studies (see section 7.8.1) cannot release TBBPA-DBMPE (or its impurities) from the suspicion of endocrine activity. No tests were conducted that are appropriate to the very low water solubility of TBBPA-DBPE or TBBPA-DBMPE.

Furthermore, it is possible that TBBPA-DBPE and TBBPA-DBMPE can be metabolised in organisms, e.g. in fish under anaerobic conditions and TBBPA or another endocrine active

substance may be formed. TBBPA is under substance evaluation because of potential endocrine disruptive properties.

The concern for TBBPA-DBPE and TBBPA-DBMPE is not resolved as the substance is very persistent and due to the super hydrophobic (Log Kow > 10) property a very slow uptake and clearence kinetic can be expected. Reaching the steady state concentration can last years. Hence, accumulation in the environment and slow accumulation in organisms is possible. In organisms endocrine active degradation products might be formed. However, at the time it is not possible to examine this.

Therefore, the eMSCA for the time being can neither conclude on the ED properties of both substances not dismiss the concern based on the difficulty to request appropriate tests.

For TBBPA -the central building block for both substances- and their potential degradation product in the environment, a substance evaluation is currently ongoing which aims to clarify TBBPA's potential for ED properties in the environment and its PBT/vPvB properties. Based on the outcome of the substance evaluation of TBBPA, the need for further testing for TBBPA-DBPE and TBBPA-DBMPE may need to be reassessed.

7.11 PBT and VPVB assessment for both Substances

Persistence

As no degradation data on TBBPA-DBPME is available cross reference is made to TBBPA-DBPE. TBBPA-DBPE is not readily biodegradable and very persistent in soil. It did not degrade in four different soils of different characteristics and accordingly no metabolites were found. DT50 soil is > 120 days.

Bioaccumulation

Based on the $logK_{ow}$ TBBPA-DBPE and TBBPA-DBMPE are screened as potential B/vB. The indicators for limited bioaccumulation are fulfilled and subsequently point to a low bioaccumulation potential. No experimental BCF or BMF data are available for TBBPA-DBMPE. Therefore, the experimental data of the read across substance TBBPA-DBPE were considered. The available BCF study for TBBPA-DBPE with carp is not reliable. Available BCF studies with earthworm, amphipods and non-biting midge indicate surface sorption instead of uptake into the organisms. Nevertheless, available monitoring data and laboratory studies indicate that TBBPA-DBPE can be taken up into organisms. As the substances are super hydrophobic (Log Kow > 10) a very slow uptake and clearence kinetic can be expected and reaching the steady state concentration can last years (Larisch and Goss, 2018). Subsequently there is a concern for slow bioaccumulation which is actually not covered by the guidance and therefore more research is needed for this developmental issue.

Based on the actual available guidance R.11 (2017) and the available data for TBBPA-DBPE and TBBPA-DBMPE we conclude that it seems unlikely that the substances are bioaccumulative in organisms.

Toxicity

Tests with TBBPA-DBPE: No effects were seen in acute fish tests. Also a mesocosm study with fish (but with very small sample size and hence very high standard deviation) and a short term reproduction assay (prolonged for offspring) did not show effects. Two Fish embryo tests were also negative.

Either no effects were seen for invertebrates in the acute Daphnia test (48h). No effects appeared in the algae toxicity test. In a sediment prolonged toxicity study with *Hyalella Azteca* (28 d, OPPTS 850.1735 (draft 1996)) an effect was seen on length (NOEC of 642 mg/kg), weight and survival were unaffected. A sediment test with *Chironomus riparius* (28 d, OECD TG 218) did not show effects (NOEC is >= 810 mg/kg dry sediment) but was considered to be less reliable since the chironomids received uncontaminated food.

No effects appeared in the algae toxicity test.

In a prolonged earthworm reproduction test (56 d for reproduction, 28 d for mortality and weight) an effect was seen on reproduction at 1024 mg/kg soil dw, NOEC = 512 mg/kg soil dw. No effects appeared regarding mortality and biomass.

Test with TBBPA-DBMPE: No effects were seen in an acute Daphnia study.

In the available tests on both substances, either no effects were seen or effects only appeared at high concentrations. But knowing that TBBPA-DBPE and TBBPA-DBMPE are very slowly taken up by organisms (reaching the steady state concentration can last years), it has to be reasonably assumed that the concentrations in the organisms are very low. Unfortunately the concentrations in the organisms were not measured. Nevertheless, effects on reproduction in earthworm and effects on length in *Hyalella azteca* were seen.

In summary, effects were either not seen or only at high test concentrations.

Overall conclusion on PBT/vPvB properties

In summary, TBBPA-DBPE and TBBPA-DBMPE are very persistent. They are highly hydrophobic with reaching steady state requiring years. Bioaccumulation criteria according to actual available guidance are not fulfilled. No toxicity to environmental organisms occurred or only at high concentrations. Therefore, these substances are not considered as PBT/vPvB by the eMSCA.

7.12 Exposure assessment

7.12.1 Human health for both Substances

Not assessed during this evaluation.

7.12.2 Environment

TBBPA-DBPE

The substance is manufactured and/or imported in the European Economic Area in 1 000 - 10 000 tons per year. The substance is used in articles, in formulation or re-packing and at industrial sites.

The substance is used for the manufacture of plastic products, textile and leather or fur. Other fields of application are dyes, pH-regulators and water treatment products. Release to the environment of this substance is likely to occur from industrial use in the production of articles, in processing aids at industrial sites and as processing aid.

High release rates could be expected during outdoor use in long-life materials (e.g. tyres, treated wooden products, treated textile and fabric, brake pads in trucks or cars, sanding of buildings (bridges, facades)) or vehicles (ships) and indoor use in long-life materials with high release rate (e.g. release from fabrics, textiles during washing, removal of indoor paints).

Other release to the environment of TBBPA-DBPE is likely to occur from indoor use in long-life materials with low release rate (e.g. flooring, furniture, toys, construction materials, curtains, foot-wear, leather products, paper and cardboard products, electronic equipment). Furthermore, the substance can be found in complex articles, with no release intended. For instance vehicles and machinery, mechanical appliances and electrical/electronic products (e.g. computers, cameras, lamps, refrigerators, washing machines). The substance can be found in products with material based on fabrics, textiles and apparel (e.g. clothing, mattress, curtains or carpets, textile toys) and plastic (e.g. food packaging and storage, toys, mobile phones).

According to the notifications provided by companies in REACH registrations no hazards have been classified. Therefore, no exposure assessment for the different environmental compartments (soil, surface water, sediment, air etc.) has been carried out by the registrants. Against this backdrop, no PEC- values for the environment are available so far.

Nevertheless, reviewing the literature there are findings of TBBPA-DBPE in various environmental compartments as well as in biota. TBBPA-DBPE has been found in the environment in Asia and Europe as well as in the North American ambient environment. Irrespective of the fact that TBBPA-DBPE exhibits a high molecular weight of 943.61 g/mol and a low solubility in water (0.14µg/L) as well as a low volatility (0.02 Pa), the substance can be detected in the aquatic as well as in the terrestrial and atmospheric compartment (**Error! Reference source not found.**). In a few studies the concentration of TBBPA-DBPE in soil, sediment and sewage sludge from China are reported (Shi et al., 2009), (Qu et al., 2013). The substance has been also detected in house dust samples from California (Dodson et al., 2012) as well as in samples from Belgium and the United Kingdom (Ali et al., 2011). (Nyholm et al., 2013) reported on TBBPA-DBPE in the downstream of a waste water treatment plant and in seepage water of a metal recycling factory in Norway. (Liu et al., 2016a) measured TBBPA-DBPE concentrations by high-volume air sampling in the particle samples taken in the Great Lakes area in the USA.The authors concluded that this observation suggests that this substance might be transported for considerable distances in association with small particles.

Table 18

Occurrence of TBBPA-DBPE in environmental compartments							
Literature	**Soil [ng/g dw]**	**Sediment [ng/g dw]**	**Air [pg/m^3]**	**WWTP [ng/L]**	**Sewage [ng/g dw]**	**Indoor dust [ng/g]**	**Seepage Water [ng/L]**
Shi et al., 2009	17 - 60	1.5 - 2 300	130 - 1 240		240 - 8 950		
Nyholm et al., 2013				18			81
Qu et al., 2013	25 - 85	2 500					
Ali et al., 2011						20 – 9 960	
Liu et al., 2016a			0.19 - 1.3				
Dodsen et al., 2012						10 - 560	

TBBPA-DBPE has been found in various fish species from Southern China and Norway (Shi et al., 2009), (Liu et al., 2016b), (Sagerup et al., 2010). (Qu et al., 2013) reported on its concentrations in mollusks, earthworm and birds from the Bohai Sea in China. (Shi et al., 2009) measured TBBPA-DBPE in fish and bird samples from an electronic waste processing area in Southern China. The measurement of the substance in Herring Gull egg samples from colonial locations in the St. Lawrence River and Great Lakes Basins in Canada demonstrates its bioavailability (Letcher and Chu, 2010). The observations made for TBBPA-DBPE in various biota are in line with the appearance of other emerging brominated flame retardants and their degradation products (e.g. Tetrabromobishenol A (TBBPA) Cluster) in the environment.

TBBPA-DBMPE

The substance is manufactured and/or imported in the European Economic Area in 100 – 1 000 tons per year. The substance is used in articles, in formulation for re-packing and at industrial sites.

The substance is used for the manufacture of plastic products, furniture, textile and leather or fur. Release to the environment of the substance is likely to occur from formulation of mixtures and formulation into materials at industrial site. For instance TBBPA-DBPME is applied in polymers and textile treatment products as well as in dyes.

During indoor use in long-life materials (e.g. flooring, furniture, toys) a low release rate is expected. Other release to the environment of TBBPA-DBPE is likely to occur from outdoor use in long-life materials with low release rate (e.g. metal, wooden and plastic construction and building materials). Furthermore, the substance can be found in complex articles, with

no release intended. For example electrical batteries and accumulators. The substance can be found in products with material based on fabrics, textiles and apparel (e.g. clothing, mattress, curtains or carpets, textile toys) and plastic (e.g. food packaging and storage, toys, mobile phones) as well as rubber (e.g. tyres, shoes, toys). In view of the applications of TBBPA-DBPME foreseen, a wide dispersive use can be postulated.

According to the notifications provided by companies in REACH registrations no hazards have been classified. Therefore, no exposure assessment for the different environmental compartments (soil, surface water, sediment, air etc.) has been carried out by the registrant. Against this backdrop, no PEC values for the environment are available so far.

Reviewing the literature, there are no reports on the occurrence of TBBPA-DBPME in environmental compartments and biota. Nevertheless, due to the closely related structural similarities of the substance compared to TBBPA-DBPE, its physical-chemical properties and the intended uses the same conclusions on the emission pattern and exposure concern on TBBPA-DBPME can be drawn.

7.12.3 Combined exposure assessment for both Substances

No combined exposure assessment for TBBPA-DBPE and for TBBPA-DBPE TBBPA-DBPME has been carried out by the registrants. Consequently, a combined exposure assessment due to aggregated tonnages and combined uses from different registrants is not presented here.

7.13 Risk characterisation

Environment

Due to the lack of any predicted environmental concentrations (PEC's) for the environmental compartments a risk characterization for TBBPA-DBPE and TBBPA-DBMPE based on risk quotients (RCR-values) can not be presented here. Nevertheless, due to the persistence and the intended uses of the substances, especially the long lasting article service life (e.g. plastics), a continuous release of TBBPA-DB(M)PE to the environment raises exposure concern.

Formulation

The substances are used in closed processes during the preparation of polymers. However, since the substances are not covalently bound to the polymer matrix a continuous release to man and environment during the article service life can be expected.

Uses at industrial sites

The environmental release categories are pointing to a possible wide dispersive exposure of the environment via these uses as flame retardants in plastic articles.

Article service life for TBBPA-DBPE

The ERC's provided by the registrants are ERC 10a, 10b, 11a and 11b which point to wide dispersive outdoor and indoor use of long life plastic articles with low as well as high releases. However, especially the wide dispersive outdoor use combined with the very high persistency of TBBPA-DBPE raises exposure concern for environmental compartments.

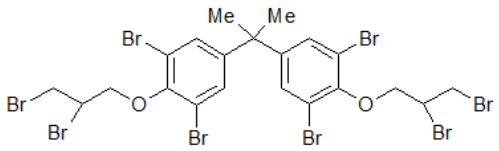
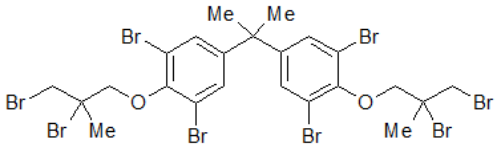
Article service life for TBBPA-DBMPE

The ERCs provided in the registration are ERC 10a and ERC 11a pointing to wide dispersive outdoor and indoor use of long life plastic articles with low releases. However, especially the wide dispersive outdoor use of TBBPA-DBPME combined with the very high persistency of the read across substance TBBPA-DBPE raises exposure concern for environmental compartments.

7.14 Read across from TBBPA-DBPE to TBBPA-DBMPE

In view of the same basic chemical structure the only differenc between TBBPA-DBMPE and TBBPA-DBPE is that TBBPA-DBMPE has an additional methyl group at each side chain.

Table 19

SUMMARY DATA ON IDENTIFICATION, PHYSICAL AND CHEMICAL PROPERTIES OF TBBPA-DBPE AND TBBPA-DBMPE		
Parameters	**TBBPA-DBPE**	**TBBPA-DBMPE**
Chemical structure		
SMILES	CC(C)(C1=CC(Br)=C(OCC(Br)CBr)C(Br)=C1)C1=CC(Br)=C(OCC(Br)CBr)C(Br)=C1	CC(Br)(CBr)COC1=C(Br)C=C(C=C1Br)C(C)(C)C1=CC(Br)=C(OCC(C)(Br)CBr)C(Br)=C1
Molecular formula	$C_{21}H_{20}Br_8O_2$	$C_{23}H_{24}Br_8O_2$
Molecular weight (g/mol)	943.624	971.667
Physical state at 20 °C and 101.3 kPa	Solid, white crystalline powder (visual examination)	Solid, White powder (visual examination)
Relative density	0.96 g/cm^3 (20 °C)	0.7 - 0.9 (Data derived from expert judgement)
Melting point	107.3 °C (Optical melting determination) 113.39 °C (Differential scanning calorimeter)	ca. 281.21 °C (estimated by means of MPBWIN v1.43)
Vapour pressure	0.029 Pa (20°C), Static method	2.99 e-13 Pa (estimated by means of MPBWIN v1.43)
Water solubility	0.144 µg/L at 20°C (OECD TG 105 (Water Solubility)	< 20 µg/L at 20 °C (pH: >= 5.97 - <= 6.19) OECD TG 105 (Water Solubility)
Partition coefficient n-octanol/water (log Kow)	> 7.2 at 23 °C, pH = 5.7 [OECD TG 123 (Partition Coefficient (1-Octanol /Water), Slow-Stirring Method)] 11.52 (estimated by KOWWIN v1.68)	ca. 12.42 (Data estimated by means of KOWWIN v1.68, 2010)
Dissociation constant (pKa)	n.a. (study scientifically not necessary / other information available)	n.a. (study technically not feasible)

7.15 References

Ali N., Harrad S., Goosey E., Neels H., and Covaci A. (2011): "Novel" brominated flame retardants in Belgian and UK indoor dust: implications for human exposure. Chemosphere 83 (10), 1360-1365. DOI: 10.1016/j.chemosphere.2011.02.078

Austin B. (2006): The bacterial microflora of fish, revised. ScientificWorldJournal 6, 931-945. DOI: 10.1100/tsw.2006.181

Canton R.F., Sanderson J.T., Letcher R.J., Bergman A., and van den Berg M. (2005): Inhibition and induction of aromatase (CYP19) activity by brominated flame retardants in H295R human adrenocortical carcinoma cells. Toxicol Sci 88 (2), 447-455. DOI: 10.1093/toxsci/kfi325

Canton R.F., Sanderson J.T., Nijmeijer S., Bergman A., Letcher R.J., and van den Berg M. (2006): In vitro effects of brominated flame retardants and metabolites on CYP17 catalytic activity: a novel mechanism of action? Toxicol Appl Pharmacol 216 (2), 274-281. DOI: 10.1016/j.taap.2006.05.007

de Jourdan B.P. (2012): Environmental Fate and Toxicity of Three Brominated Flame Retardants in Aquatic Mesocosms, University of Guelph, Ontario, Canada

de Jourdan B.P., Hanson M.L., Muir D.C., and Solomon K.R. (2014): Fathead minnow (Pimephales promelas Rafinesque) exposure to three novel brominated flame retardants in outdoor mesocosms: bioaccumulation and biotransformation. Environ Toxicol Chem 33 (5), 1148-1155. DOI: 10.1002/etc.2541

Dodson R.E., Perovich L.J., Covaci A., Van den Eede N., Ionas A.C., Dirtu A.C., Brody J.G., and Rudel R.A. (2012): After the PBDE phase-out: a broad suite of flame retardants in repeat house dust samples from California. Environ Sci Technol 46 (24), 13056-13066. DOI: 10.1021/es303879n

Ezechias M., Svobodova K., and Cajthaml T. (2012): Hormonal activities of new brominated flame retardants. Chemosphere 87 (7), 820-824. DOI: 10.1016/j.chemosphere.2011.12.049

Hamers T., Kamstra J.H., Sonneveld E., Murk A.J., Kester M.H., Andersson P.L., Legler J., and Brouwer A. (2006): In vitro profiling of the endocrine-disrupting potency of brominated flame retardants. Toxicol Sci 92 (1), 157-173. DOI: 10.1093/toxsci/kfj187

Hamers T., Kamstra J.H., Sonneveld E., Murk A.J., Visser T.J., Van Velzen M.J., Brouwer A., and Bergman A. (2008): Biotransformation of brominated flame retardants into potentially endocrine-disrupting metabolites, with special attention to 2,2',4,4'-tetrabromodiphenyl ether (BDE-47). Mol Nutr Food Res 52 (2), 284-298. DOI: 10.1002/mnfr.200700104

Knudsen G.A., Jacobs L.M., Kuester R.K., and Sipes I.G. (2007): Absorption, distribution, metabolism and excretion of intravenously and orally administered tetrabromobisphenol A [2,3-dibromopropyl ether] in male Fischer-344 rats. Toxicology 237 (1-3), 158-167. DOI: 10.1016/j.tox.2007.05.006

Lans M.C., Klasson-Wehler E., Willemsen M., Meussen E., Safe S., and Brouwer A. (1993): Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-p-dioxins and -dibenzofurans with human transthyretin. Chemico-Biological Interactions 88 (1), 7-21. DOI: https://doi.org/10.1016/0009-2797(93)90081-9

Larisch W. and Goss K.U. (2018): Modelling oral up-take of hydrophobic and super-hydrophobic chemicals in fish. Environ Sci Process Impacts 20 (1), 98-104. DOI: 10.1039/c7em00495h

Letcher R.J. and Chu S. (2010): High-sensitivity method for determination of tetrabromobisphenol-S and tetrabromobisphenol-A derivative flame retardants in great lakes herring gull eggs by liquid chromatography-atmospheric pressure photoionization-tandem mass spectrometry. Environ Sci Technol 44 (22), 8615-8621. DOI: 10.1021/es102135n

Li D., Chen H., Mao B., Yang Q., Zhao J., Gu Z., Zhang H., Chen Y.Q., and Chen W. (2017): Microbial Biogeography and Core Microbiota of the Rat Digestive Tract. Scientific Reports 7 (1), 45840. DOI: 10.1038/srep45840

Liu A., Shi J., Qu G., Hu L., Ma Q., Song M., Jing C., and Jiang G. (2017): Identification of Emerging Brominated Chemicals as the Transformation Products of Tetrabromobisphenol A (TBBPA) Derivatives in Soil. Environ Sci Technol 51 (10), 5434-5444. DOI: 10.1021/acs.est.7b01071

Liu A.F., Qu G.B., Yu M., Liu Y.W., Shi J.B., and Jiang G.B. (2016a): Tetrabromobisphenol-A/S and Nine Novel Analogs in Biological Samples from the Chinese Bohai Sea: Implications for Trophic Transfer. Environ Sci Technol 50 (8), 4203-4211. DOI: 10.1021/acs.est.5b06378

Liu L.-Y., Venier M., Salamova A., and Hites R. (2016b): A Novel Flame Retardant in the Great Lakes Atmosphere: 3,3′,5,5′-Tetrabromobisphenol A Bis(2,3-dibromopropyl) Ether. Environ. Sci. Technol. Lett., 194-199. https://pubs.acs.org/doi/abs/10.1021/acs.estlett.6b00138

Liu Q., Ren X., Long Y., Hu L., Qu G., Zhou Q., and Jiang G. (2016c): The potential neurotoxicity of emerging tetrabromobisphenol A derivatives based on rat pheochromocytoma cells. Chemosphere 154, 194-203. DOI: 10.1016/j.chemosphere.2016.03.117

Ma M., Crump D., Farmahin R., and Kennedy S.W. (2015): Comparing the effects of tetrabromobisphenol-A, bisphenol A, and their potential replacement alternatives, TBBPA-bis(2,3-dibromopropyl ether) and bisphenol S, on cell viability and messenger ribonucleic acid expression in chicken embryonic hepatocytes. Environ Toxicol Chem 34 (2), 391-401. DOI: 10.1002/etc.2814

Newsome C.S.H., D.; Marshall, S..J.; van Egmond, R.A. (1995): Fate of Some Anionic and Alcohol Ethoxylate Surfactants in Carassıus Auratus. Tenside Surf. Det. 32 (6)

Norman Haldén A. (2010): Exposure of Zebrafish to Brominated Environmental Chemicals; Studies on reproduction, maternal transfer and early life-stage development, Swedish University of Agricultural Sciences, Uppsala

Noyes P.D., Haggard D.E., Gonnerman G.D., and Tanguay R.L. (2015): Advanced morphological - behavioral test platform reveals neurodevelopmental defects in embryonic zebrafish exposed to comprehensive suite of halogenated and organophosphate flame retardants. Toxicol Sci 145 (1), 177-195. DOI: 10.1093/toxsci/kfv044

Nyholm J.R., Grabic R., Arp H.P., Moskeland T., and Andersson P.L. (2013): Environmental occurrence of emerging and legacy brominated flame retardants near suspected sources in Norway. Sci Total Environ 443, 307-314. DOI: 10.1016/j.scitotenv.2012.10.081

Qu G., Liu A., Wang T., Zhang C., Fu J., Yu M., Sun J., Zhu N., Li Z., Wei G., Du Y., Shi J., Liu S., and Jiang G. (2013): Identification of tetrabromobisphenol A allyl ether and tetrabromobisphenol A 2,3-dibromopropyl ether in the ambient environment near a manufacturing site and in mollusks at a coastal region. Environ Sci Technol 47 (9), 4760-4767. DOI: 10.1021/es3049916

Sagerup K., Herzke D., Harju M., Evenset A., Christensen G.N., Routti H., Fuglei E., Aars J., Strøm H., and Gabrielsen G.W. (2010): New brominated flame retardants in Arctic biota. Norway, KLIMA-OG Forurensings-Directoratet

Shi T., Chen S.J., Luo X.J., Zhang X.L., Tang C.M., Luo Y., Ma Y.J., Wu J.P., Peng X.Z., and Mai B.X. (2009): Occurrence of brominated flame retardants other than polybrominated diphenyl ethers in environmental and biota samples from southern China. Chemosphere 74 (7), 910-916. DOI: 10.1016/j.chemosphere.2008.10.047

Testing Laboratory (2002a): The toxicity to Rainbow Trout (Oncorhynchus mykiss) of TBBA-Bis(2,3-dibromopropyl ether) (FR-720)

Testing Laboratory (2002b): The growth inhibition of the alga Selenastrum capricornutum by Tetrabromobishphenol A, Bis(2,3-dibromopropyl ether).

Testing Laboratory (2002c): The toxicity to Daphnia magna of Tetrabromobisphenol A, Bis (2,3-Dibromopropyl Ether) (FR-720).

Testing Laboratory (2004): Eisenia fetida reproduction test (according to ISO 11268-2). Test item: tetrabromobisphenol-A-dibromopropylether (TBBPA-DBPE).

Testing Laboratory (2005a): Sediment- water chironomid (Chironomus riparius) toxicity test using spiked sediment, Guideline: OECD 218, (February, 2001), Test item: tetrabromobisphenol-A-dibromopropylether (TBBPA-DBPE)

Testing Laboratory (2005b): Sediment prolonged toxicity study with Hyalella azteca, Guideline: OPPTS 850.1735 (1996), Test item: Tetrabromobisphenol-A-dibromopropylether (TBBPA-DBPE).

Testing Laboratory (2015): Determination of short term toxicity of AP 1300 S against Daphnia magna STRAUS according to OECD 202 resp. EU C.2 15031802G201.

Wielogorska E., Elliott C.T., Danaher M., and Connolly L. (2015): Endocrine disruptor activity of multiple environmental food chain contaminants. Toxicol In Vitro 29 (1), 211-220. DOI: 10.1016/j.tiv.2014.10.014

Yakata N. (2003): Bioconcentration study of BDBPT in carp. Kurume Laboratory Chemicals Evaluation and Research Institute Japan on behalf of FLAME RETARDANT CHEMICALS ASSOCIATION OF JAPAN

7.16 Abbreviations

AhR	Aromatic hydrocarbon receptor
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BDE	Brominated diphenyl ether
BMF	Biomigration factor
CALUX	Chemical Activated Luciferase gene eXpression
CCH	Compliance Check
CLP	Classification Labelling Packaging
CoRAP	Community rolling action plan
DMSO	Dimethylsufoxide
E2	Estradiol
EC50	Half maximal effective concentration
ED	Endocrine disruption
ERC	Environmental Release Categories
FET	Fish embryo toxicity test
GSI	Gonadosomatic index
HPLC	High pressure liquid chromatography
IUCLID	International Uniform ChemicaL Information Database
11-KT	11-Ketotestosterone
LC	Lethal concentration
LOEC	Lowest observed effects concentration
LSI	Liver somatic index
NOEC	No observed effects concentration
OPPTS	Office of Pollution Prevention and Toxics
PBT	Persistent, bioaccumulative, toxic
PCR	Polymerase chain reaction
PNEC	Predicted no effect concentration
RIVM	Rijksinstituut voor Volksgezondheid en Milieu
SVHC	Substances of very high concern
TBBPA	2,2,6,6-tetrabromo-4,4-isopropylidenediphenol (Tetrabromobisphenol A)
TBBPA-BAE	Tetrabromobisphenol A bisallylether
TBBPA-DBMPE	1,1'-(isopropylidene)bis[3,5-dibromo-4-(2,3-dibromo-2-methylpropoxy)]benzene
TBBPA-DBPE	1,1'-(isopropylidene)bis[3,5-dibromo-4-(2,3-dibromopropoxy)]benzene
TBBPA-MAE	Tetrabromobisphenol A monoallylether
TL	Testing Laboratory
UBA	Umweltbundesamt or German Environment Agency
VTG	Vitellogenin
WAF	Water accommodated fraction