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Helsinki, 23 March 2017

Substance name: 2,2´6,6´-tetrabromo-4-4´isopropylidenediphenol
EC number: 201-236-9
CAS number: 79-94-7
Date of Latest submission(s) considered[1]: 15 June 2016
Decision/annotation number: Please refer to the REACH-IT message which delivered this communication (in format SEV-C-0000006434-75-02/F)
Addressees: Registrant(s)[2] of 2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol (Registrant(s))

DECISION ON SUBSTANCE EVALUATION

1. Requested information

Based on Article 46(1) of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), you are requested to submit the following information on **the registered substance subject to this decision**:

1.1 The Larval Amphibian Growth and Development Assay (LAGDA); test method: OECD 241. The test shall include measurements of T3 and VTG as specified in Appendix 1.

Based on Article 46(1) of the REACH Regulation, you are requested to submit the following information on the **transformation product of the registered substance: monomethyl ether TBBPA (Phenol, 4,4 -(1-methylethylidene)-bis[2,6-dibromo-) (no CAS available) (see Appendix 3 for further information on substance identity)**:

1.2 A Dissociation Constants test using the OECD 112 (Dissociation Constants in Water);

1.3 A water solubility test using the EU A.6/OECD 105 (column elution) at 12°C;

1.4 A Partition Coefficient (1-Octanol/Water) using the OECD 123 (Slow-Stirring Method) at 12°C (c.f. appendix);

1.5 Simulation degradation testing: Option A or B, depending on the outcome of the information requested in 1.4 and depending on analytical possibilities:

A. If technically feasible depending on analytical possibilities (in particular most possibly if the water solubility is > 1 µg/L) the following tests shall be carried out:

[1] This decision is based on the registration dossier(s) on the day until which the evaluating MSCA granted an extension for submitting dossier updates which it would take into consideration.

[2] The terms Registrant(s), dossier(s) or registration(s) are used throughout the decision, irrespective of the number of registrants addressed by the decision.

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Simulation testing on ultimate degradation in surface water, test method: Aerobic mineralisation in surface water – simulation biodegradation test, EU C.25/OECD 309, with suspended solids/sediment particles (~15 mg SPM dw/L as specified in Appendix 1), at 12 °C. The test set-up shall enable to check the mass balance (using radiolabelled test substance). The pathway (metabolism) part of the study does not need to be conducted at this stage if the kinetic study indicates that the primary degradation half-life in surface water of monomethyl ether TBBPA is > 60 days or if the ultimate degradation half-life in surface water is < 40 days.

B. If the conduct of the study requested above under 1.5 A is not technically feasible (e.g. if not possible within reasonable scientific and technical efforts in particular if the water solubility < 1 µg/l), the following test shall be carried out as specified in Appendix 1: Sediment simulation testing (Aerobic and anaerobic transformation in aquatic sediment systems, EU C.24./OECD 308) at 12°C. The test set-up shall enable to check the mass balance (using radiolabelled test substance). The pathway (metabolism) part of the study does not need to be conducted at this stage if the kinetic study indicates that the primary degradation half-life in sediment of monomethyl ether TBBPA is > 180 days or if the ultimate degradation half-life in sediment is < 120 days.

Based on Article 46(1) of the REACH Regulation, you are requested to submit the following information on the **transformation product of the registered substance**: **bismethyl ether TBBPA (CAS 37853-61-5) (see Appendix 3 for further information on substance identity**.

1.6 Soil simulation testing (test method: Aerobic and anaerobic transformation in soil, EU C.23./OECD 307) at 12 °C (as specified in Appendix 1). The test set-up shall enable to check the mass balance (using radiolabelled test substance). The pathway (metabolism) part of the study does not need to be conducted at this stage if the kinetic study indicates that for bismethyl ether TBBPA the primary degradation half-life in soil is > 180 days or if the ultimate degradation half-life in soil is <120 days.

For all studies requested above the evaluating MSCA must have access to the full study reports including all relevant details of the studies, ensuing that a clear conclusion can be drawn by the evaluating MSCA.

You shall provide an update of the registration dossier(s) containing the requested information, including robust study summaries and, where relevant, an update of the Chemical Safety Report by **4 January 2021**. The deadline takes into account the time that you, the Registrants, may need to agree on who is to perform any required tests. It has been set to allow for sequential testing or other sequential information gathering or information generation approaches as appropriate.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Further information, observations and technical guidance as appropriate are provided in Appendix 3. Appendix 4 contains a list of registration numbers for the addressees of this decision. This Appendix is confidential and not included in the public version of this decision.

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2. Who performs the testing

Based on Article 53 of the REACH Regulation, you are requested to inform ECHA who will carry out the studies on behalf of all Registrants within 90 days. Instructions on how to do this are provided in Appendix 3.

3. Appeal

You can appeal this decision to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, shall be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under http://echa.europa.eu/regulations/appeals

Authorised[3] by Leena Ylä-Mononen, Director of Evaluation

[3] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

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Appendix 1: Reasons

Based on the evaluation of all relevant information submitted on 2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol available in IUCLID and other relevant available information from the open literature up until 18 December 2015, ECHA concludes that further information is required in order to enable the evaluating Member State Competent Authority (MSCA) to complete the evaluation of whether the substance constitutes a concern in respect to the chemical safety for human health or the environment.

The evaluating MSCA will subsequently review the information submitted by you and evaluate if further information should be requested in order to clarify the concern for endocrine disrupting properties, exposure and PBT properties/environmental fate of methylated transformation products.

For each of the areas of concern, further information is being requested now. An alternative option to this parallel testing strategy regarding the unrelated areas of concern for PBT properties of certain degradation products and endocrine disruptive properties of the registered substance would be to request further information for clarification of either endocrine disruption or for PBT properties first in a sequential testing strategy. Since investigation of PBT-properties, however, is stepwise and may take several years, such a sequential testing strategy would cause an unacceptable delay in the clarification of concerns, as outlined below. This is evaluated to be an inappropriate way forward due to the seriousness of these endpoints and the following urgency of clarifying the concerns as quickly as reasonably possible.

It has therefore been concluded that it is proportionate to request the above mentioned further information for each of the areas of concern in parallel now in order to clarify these concerns.

In regards to PBT, the requests for more information about the persistency (in this case on two transformation products of the registered substance) follows the general PBT testing strategy which in itself is sequential, meaning that several steps, each resulting in new information requests, might have to take place before being able to conclude on this area of concern. This will take a considerable amount of time as the request for further information about the PBT properties focusing on persistency of two transformation products will only be conclusive if the information provides evidence of these transformation products not being persistent. If not so, further information will be requested in respect to the bioaccumulation potential and potentially hereafter on the chronic toxicity towards aquatic organism and/or mammalian species.

It should be noted that the concerns for PBT properties do not lead to requests for animal testing at this initial stage and hence, in respect to laboratory animal welfare, there is no issue related to the present requests. As further specified in Endpoint 5 and 6 both transformation products meet the PBT screening criteria and both transformation products have been detected in the environment.

It is a priori not possible to predict exactly how similar the degradability of the two transformation products in different environmental compartments will be even though they are closely structurally related. Therefore it is necessary to determine the persistency experimentally for both transformation products in parallel to obtain degradation half-lives for both, which can be compared with the P/vP criteria of REACH Annex XIII. Based on this it can be decided for which transformation product(s), if any, the next step in the PBT assessment would be warranted. Nevertheless, in the case one of the transformation products is shown to be very persistent, it might be justified by

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employing a cautious approach to draw the same conclusion for the other transformation product without further simulation degradation testing.

In regards to endocrine disruption, the current decision focuses on clarifying the concern for endocrine disruption to non-mammalian vertebrate wildlife.
At this stage, the outcome of the investigations of endocrine disruption of TBBPA and regarding the PBT properties (including the degradability as initially requested to be tested) of the two identified relevant transformation products of TBBPA are unknown. If a sequential approach in respect to both ED and PBT properties would be chosen, and the first area of concern to be investigated (PBT or ED) shows out to be of no concern, the next step would be to move on to investigate the next area of concern (i.e. ED if PBT has first been considered and vice versa), which would then be investigated with an unnecessary delay.

Both above mentioned areas of concerns (PBT and ED) could, depending on the results of the requested information, now or in the future possibly lead to a decision of the evaluating MSCA to propose the substance for inclusion on the Candidate List under REACH article 57 (d), (e) or (f) as appropriate based on PBT, vPvB or endocrine disruptive properties, respectively. However, it is not certain that the risk management measures for these scenarios will be the same, as a non-threshold type of assessment and regulation are applied for substances meeting the criteria of Article 57(d) or (e) where the relevance of a threshold approach for substances meeting the criteria of article 57(f) remains to be clarified.

Consideration of the time needed to perform the requested studies

You proposed an extension of the test deadline from 27 to 45 months based on a number of arguments, including the complexity of synthesis of the monomethyl ether TBBPA and analytical difficulties. A deadline of 45 months is now granted.

You argued in your comments that a timeframe of 15 month for the performance of the LAGDA test would be far too short as it, in your opinion, is a new complex guideline with limited capacity of laboratories experienced in performing it. You further argued that for requested studies on PBT-properties the timeframe of 36 months was too short based on complexity of studies and the analytical difficulties foreseen. You highlighted that this was based on the assumption that the process of the performance of the requested simulation studies would be a parallel approach. You stated that you do agree with the Proposal for Amendment (PfA) from a MSCA that a parallel testing scheme is not warranted and that the testing of the persistence of the monomethyl ether TBBPA and the bismethyl ether TBBPA should be performed sequentially. You suggested that the persistence of the bismethyl ether TBBPA could be investigated, if at all, only if the monomethyl ether TBBPA is found not to be persistent. You claimed that assuming a tiered or sequential approach for testing the two transformation products, a 45 month timeline is more realistic rather than 36 month. You further claimed that no additional time for the time consuming dossier update and the respective evaluations have been given, which can only be performed when all information is available.

ECHA highlights that the final deadline is based on the longest duration of the requested test-design thus the request concerning reporting of the results of the LAGDA will not have a separate deadline of 15 months. ECHA does not find it proportional to test the two transformation products in a tiered or sequential approach. A conditional testing strategy where bismethyl ether is tiered to follow the persistence testing of monomethyl ether TBBPA, would mean an additional 27-33 months (depending on whether a parallel

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or sequential testing strategy is used to test the persistency of bismethyl ether TBBPA) if included in the same decision. ECHA does not regard it proportionate to add 2-3 years to the timeframe of investigation of the identification of a PBT/vPvB substance, which is already sequential and may last considerably more than 10 years all together. Both transformation products meet the screening criteria for further PBT assessment information requests (see Endpoint 5 and 6) and should, for the reasons explained above, be tested in parallel. ECHA further notes that this parallel testing of persistence of the two suspected PBT transformation products does not involve animal testing. Due to an additional request by an MSCA an additional 6 months was added to the final reporting deadline.

You also supported the PfA from a MSCA that since both the monomethyl ether TBBPA and the bismethyl ether TBBPA are minor degradants of TBBPA, it is likely that secondary degradants may be near or below the 0.1% threshold for PBT assessment the registered substance. A request to further identify those degradation products is therefore not warranted and disproportionate in your view.

ECHA highlights that even though it is not possible to quantify the formation of the transformation products precisely the formation of the two transformation products from TBBPA could be as high as 10-60%.

You also supported a statement made by a MSCA that there was no indication in the draft decision nor in data from industry that the bismethyl ether TBBPA is formed in amounts that could cause concern. Based on this you argued that therefore monomethyl ether TBBPA should be tested first and bismethyl ether TBBPA, if relevant, should be tested later.

However, the evaluating MSCA has identified a PBT-concern for both transformation products and hence ECHA does not find it proportional to add another extra 2-3 years to clarify the persistence of the two transformation products in a testing strategy (PBT) which is already sequential (first P, then B, and the T if relevant). However, based on this there is an opportunity to extrapolate persistency data for one transformation product to another if considered relevant (further information under Endpoint 5 and 6).

ENDPOINT 1 Endocrine disruptive properties: The Larval Amphibian Growth and Development Assay (LAGDA); test method: OECD 241 using the registered substance.

The Concern(s) Identified

The evaluating MSCA has identified a concern for effects of TBBPA on the thyroid hormone system. This concern is based on various studies including both *in vitro* and *in vivo* studies.

A vast number of mechanistic studies have been performed that indicate significant effects of TBBPA on key events / processes involved in the thyroid hormone system in a range of *in vitro* assays and *in vivo* studies in various vertebrate animal species. The most marked and consistent effect of TBBPA is its ability to work as a very potent competitor of T4 binding to TTR (Meerts *et al.,* 2000; Legler *et al.,* 2002; Hamers *et al.,* 2006) with a higher affinity than the natural ligand. A new study (Iakovleva et al. (2016) confirms the high binding affinity of TBBPA to TTR and even concludes that: "*TBBPA binds TTR with an extremely high selectivity in human plasma*". The high TTR-binding potency of TBBPA indicates that this step of thyroid hormone signalling pathway might be one of the critical effects of TBBPA. Some studies have shown that TBBPA has no affinity for the TR (Kitagawa *et al.,* 2003; Kitamura *et al.,* 2005a; Hamers *et al.,* 2006;

Levy-Bimbot *et al.,* 2012) whereas others have shown that TBBPA may have both agonistic and in particular antagonistic effects, in the presence of T3 (Kitamura *et al.,* 2002; Hofman *et al.,* 2009; Freitas *et al.,* 2011; Terasaki *et al.,* 2011; Fini *et al.,* 2012a, b). A possible explanation could be that TBBPA works as a partial agonist. A partial agonist can display both agonistic and antagonistic effects when both a full agonist (T3) and partial agonist are present. The mechanism is that the partial agonist acts as a competitive antagonist in the *in vitro* assay by competing with the full agonist for binding to the receptor, decreasing the response observed with the full agonist alone. One study has also shown TBBPA to be a potent inhibitor of deiodinase (DI) activity (Butt *et al.,* 2011). These thyroid hormone related mechanistic *in vitro* effects might contribute to the overall antagonistic activity of TBBPA against the thyroid hormone signalling seen *in vivo*.

Possible interaction of TBBPA with the thyroid hormone system of fish has been evaluated by analysis of thyroid hormones, thyroid histology and genes involved in the hypothalamic-pituitary-thyroid (HPT) axis. In European flounder exposed to TBBPA, levels of the thyroid hormone thyroxin (T4) increased with internal concentrations of the test compound (Kuiper *et al.*, 2007). Triiodothyronin (T3) levels were not affected and histology showed no signs of altered thyroid gland activity. In zebrafish larvae, TBBPA demonstrated significant upregulation of three genes involved in the hypothalamic-pituitary-thyroid (HPT) axis: thyroid receptor α, thyroid stimulating hormone specific β subunit, and transthyretin (TRα, TSHβ, and TTR, respectively) (Chan & Chan, 2012). In zebrafish embryos, TBBPA significantly induced TRα and reduced TSHβ genes. No effect was in this study observed on sodium iodide symporter, thyroglobulin, thyroid peroxidase or thyroid receptor β. The results from the few studies on interaction of TBBPA with the hypothalamic-pituitary-thyroid (HPT) axis of fish lead to some concern for effects on thyroid hormone signalling. For example Kuiper et al. (2007) observed increased T4 correlating with internal concentrations of TBBPA, possibly indicating competition of TBBPA for plasma protein binding and Chan & Chan (2012) analysed the effect of TBBPA on three genes involved in the HPT axis in zebrafish embryo and larvae. They found upregulation of all three genes (TRα, TSHß, and TTR) in larvae. In embryo, TBBPA significantly induced TRα and reduced TSHß genes expression.

The effects of TBBPA on amphibians have been examined in a number of studies. Overall, the vast majority of the studies on amphibians demonstrate TH antagonism of TBBPA both at the gene transcriptional level and at the morphological level.

Amphibian metamorphosis has been used as a model to reveal TH signalling disrupting activity of TBBPA as it is well-known that amphibian metamorphosis is regulated by thyroid hormones. In a 6-day T3 induced metamorphosis assay using premetamorphic tadpoles Zhang *et al.* (2014) showed that TBBPA in the range of 5.44-544 µg/L exhibited inhibitory effects on T3 induced *X. laevis* metamorphosis in terms of multiple morphological changes, including forelimb protrusion and growth, hindlimb growth, head decrease due to gill resorption, lower jaw protrusion, and abdomen shrinkage due to intestinal remodelling. This study used 2-3 replicates and repeated the experiments 2-3 times. Similarly, Fini *et al.* (2012b) found that 544 µg/L TBBPA inhibited a TH induced decrease in head area due to gill resorption in *X. laevis*. Fini *et al.* (2012b) also find that TBBPA and not its metabolites interferes with thyroid signalling in amphibians, both in the head area study (repeated 2 times) and in a TH-responsive Green Fluorescence Protein (GFP) assay (repeated 3 times). Kitamura *et al.* (2005) and Goto *et al.* (2006) reported that TBBPA suppressed T3 induced tail shortening of *Rana rugosa* tadpoles. TBBPA also inhibited spontaneous *S. tropicalis* metamorphosis controlled by endogenous circulating TH (Goto *et al.*, 2006). Jagnytsch *et al.* (2006) reported that 500 µg/L TBBPA

inhibited spontaneous metamorphosis of *X. laevis* in a 21-day metamorphosis assay. However, at this dose tadpoles showed abnormal swimming behaviour and reduced feed uptake during the first 10 days of exposure (but not during the rest of the exposure), suggesting that systemic toxic side effects occurred in part of the exposure period. The survival rate of the tadpoles was, however, not affected.

Thus five studies have shown TH antagonistic effects in amphibian metamorphosis assays. The study of Zhang *et al.* (2014) showed that TBBPA disrupts TH dependent development in a developmental stage-dependent manner. Whereas 100−1000 nM TBBPA in the spontaneous metamorphosis assay promoted *X. laevis* development from stage 51 to 56, only 10 nM inhibited development from stage 57 to 66. The authors inferred that TBBPA could agonize TH actions and promote metamorphic development when tadpoles have low levels of endogenous TH, whereas it might antagonize TH actions and inhibit metamorphic development when tadpoles have high levels of endogenous TH. Thus, the effects of TBBPA on metamorphic development might depend on the endogenous TH levels in tadpoles. These findings that TBBPA exhibited an antagonistic effect on TH actions in the presence of high TH levels, but an agonistic activity in the presence of low TH levels are further supported by data at the transcriptional level. A recent study by Zhang *et al.* (2015) found a weak TH agonistic activity for TBBPA in the absence of T3, whereas a TH antagonistic activity was found for TBBPA at higher concentrations in the presence of T3 in a screening assay based on TH-response gene expression analysis in the black-spotted frog (*Pelophylax nigromaculatus)*. Jagnytsch *et al.* (2006) reported that short-term exposure to 100−500 µg/L TBBPA antagonized TH-induced TRβ and TH responsive basic leucine zipper transcription factor (TH/bZIP) expression in *X. laevis* head tissues, whereas TBBPA alone induced expression of these TH responsive genes. Other studies have also examined the effects of TBBPA on TH responsive genes in amphibians. The majority of these studies show TH antagonistic effects of TBBPA. Zhang *et al.* (2014) demonstrated antagonistic effects of TBBPA on T3 actions in expression studies of TH response genes, including TRβ, BTEB, ST3, DIO2, and MMP2, in the intestine and hindlimb of *Xenopus laevis*. Exposure of transgenic *Xenopus* tadpoles to T3 induced a marked expression of EGFT gene, while the addition of TBBPA blocked this EGFP expression in a dose-dependent manner (Goto *et al.*, 2006). TBBPA inhibited the T3 dependent fluorescent signal in transgenic *Xenopus laevis* embryos stage NF45 bearing a TH/bZIP-eGFP construct (Fini *et al.*, 2007). In a later study using the same species, Fini *et al.* (2012a) found that TBBPA modulated the expression of TH target genes implicated in neural stem cell function or neural differentiation. Tadpoles exposed to 5.4 µg/L TBBPA showed an increase in TH mediated expression of gelatinase B mRNA within 48 h in the tail of tadpoles of the Pacific tree frog (Veldhoen *et al.*, 2006). Treatment with 54 µg/L TBBPA resulted in increased TH mediated thyroid hormone receptor alpha mRNA expression in the tadpole brain and reduced levels of PCNA transcript in the tail. TBBPA was also found to alter the mRNA abundance of thyroid hormone receptor alpha in tail, gelatinase B in brain, and PCNA in both tissues of premetamorphic tadpoles. Hinther *et al.* (2010) reported no effect of 5.44-544 µg/L TBBPA on two TH responsive gene transcripts, TH receptor β and the *Rana* larval keratin type I in a cultured tadpole tail fin biopsy. However, the lack of effect in this assay could be explained by the low responsivity of tail tissue to TH. Several studies thus show that normal thyroid hormone-mediated gene expression profiles can be significantly altered in tadpoles after exposure to low concentrations of TBBPA.

Overall, the vast majority of the studies on amphibians demonstrate TH antagonism of TBBPA both at the gene transcriptional level and at the morphological level. However, non-monotonic dose-response curves have been observed in the studies of Zhang *et al.*

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(2014; 2015) showing TH agonism at the lower concentrations of TBBPA. It was shown by radiolabelled ^{14}C-TBBPA that the antagonistic effects on T3-induced metamorphotic parameters were caused by TBBPA itself and not the 4 identified metabolites (Fini *et al.*, 2012b). Generally, systemic toxicity was not the cause of the effects on T3-induced metamorphotic parameters because TBBPA without T3 co-exposure did not cause these effects (Kitamura *et al.*, 2005; Goto *et al.*, 2006; Fini *et al.*, 2012b, Zhang *et al.*, 2014).

From the repeated dose studies in rats, it has been observed that T4 reductions occurs after exposure to TBBPA doses of 100 mg/kg bw/day and above, irrespective of exposure duration ([redacted]; van der Ven *et al.*, 2008; NTP, 2014b). In these same studies effects on T3 have been more varying, as high-dose males from a 28- RDT day study (300 mg/kg bw/day) showed increased T3 levels (van der Ven *et al.*, 2008), high-dose males from the two-generation study (1000 mg/kg bw/day) showed decreased T3 levels (MPI, 2002b) whereas no effect on T3 levels and TSH levels was the most common finding in adult TBBPA exposed animals ([redacted]; Van der Ven *et al.*, 2008; NTP, 2014a). In these studies in adult rats, thyroid weights and thyroid gland histopathology were unaffected by TBBPA exposure ([redacted]; NTP, 2014a, b; van der Ven *et al.*, 2008; [redacted]), and a 2-year carcinogenesis study in Wistar Han rats has shown no thyroid follicular hyperplasia (NTP, 2014a). In a subchronic toxicity study in mice, TBBPA did not produce any effects on the thyroid hormone system (NTP, 2014).

Given the absence of effects on TSH levels and thyroid histology in most of the performed rodent *in vivo* studies, the mechanism by which TBBPA decreases in T4 levels is still not fully understood. In the two-generation rat study, the authors suggested that the decrease could be a result of induction of hepatic T4-uridine diphosphate glucuronyl transferase (UDP-GT), the enzyme involved in the removal of T4 circulating in the blood stream. However, the hepatic enzyme levels / activities were not measured and there was no change in liver morphology. Hence the basis for the hypothesis that induction of T4 UDPGT is the cause of the decrease in T4 blood levels after exposure of rats to TBBPA is weak.

TBBPA has not been reported to induce hypothyroxinemia during postnatal development (Saegusa *et al.* 2009; [redacted]), but since decreases in T4 levels in adult rats are very well documented, it cannot be excluded that hypothyroxinemia could also occur in pregnant rats. This would result in low T4 levels during fetal life, and could consequently alter brain development.

Data from some studies investigating developmental neurotoxicity of TBBPA are present, and although the results are ambiguous, some of them could be interpreted as showing adverse effects of TBBPA exposure on brain development. Especially data from two unpublished study reports ([redacted]) investigating neurobehavioral endpoints in developmentally exposed rats, show alterations in TBBPA exposed animals. The indications of altered behaviour in these two studies were in 2006 not strong enough for the EU RAR to conclude on the developmental neurotoxicity of TBBPA, especially since the only other *in vivo* studies investigating DNT performed at that time, did not show any adverse effects (Eriksson *et al.*, 2001; Eriksson *et al.*, 1998). In these studies NMRI mice were administered 0.75 or 11.5 mg TBBPA/kg-bw orally once on PND 10 and various neurobehavioral measures were investigated, but none were affected (Eriksson *et al.*, 2001; Eriksson *et al.*, 1998). However, studies indicating that TBBPA may affect some aspects of brain development have been published since 2006, as summarised below.

After a similar exposure to the one used by Eriksson *et al.* (1998, 2001), Viberg and Eriksson (2011) reported for example biochemical changes related to cholinergic effects in the frontal cortex of neonatal NMRI mice treated once with 11.5 mg TBBPA/kg, and in a dietary developmental toxicity study Saegusa *et al.* (2009, 2012) also found effects that could suggest alterations in neuronal migration. They administered TBBPA to Sprague Dawley rats from GD 10 through PND 20 and found an increased number of reelin expressing interneurons in the dentate hilus at the highest dose (~ 800 mg/kg-bw/day), however the exact biological significance of this finding is not yet known. (Saegusa et al. 2009, 2012). As developmental hypothyroidism was not observed in this study, the authors themselves suggested that the changes could be a direct effect of TBBPA exposure on the developing brain rather than mediated through changes in thyroid hormone levels.

In a one-generation study in rats using a wide range of TBBPA doses throughout development and adulthood (Lilienthal *et al.,* 2008) no significant effects were seen in the sweet preference test, or on context or cue conditioned fear. The authors did, however, see effects of TBBPA exposure on brainstem auditory evoked potential. Based on their results they concluded that the outcome pattern suggested a predominant cochlear effect of TBBPA in females while in males neural effects were more apparent. Nakajima *et al.,* (2009) investigated behavioural changes in 3-week old mice, in the open field, contextual fear conditioning and y-maze test. The animals were treated once, 3 hours before testing, and the authors found significant effects on behaviour in the two low dose groups (0.1 and 5 mg/kg) but not in the high one (250 mg/kg). In the two lowest dose groups also high amounts of TBBPA were detected in the striatum, whereas almost no TBBPA was seen in the other examined brain regions. In the high dose group equal amounts of TBBPA were accumulated in all brain regions. The authors proposed that a compensation mechanism, as seen after exposure to higher doses of lead, could possibly account for the lack of monotonic dose-response relationship. However, as the results from this study are equivocal, it is also possible that the high dosing volumes could have influenced the results of the test

Several *in vitro* neurotoxicity studies, most published after 2006, have examined the potential for TBBPA to affect cellular function. TBBPA has been shown to induce cytotoxicity in various neuronal cell types at doses ranging from 15-25 µM (Qu *et al.*, 2011; Ziemińska *et al.*, 2012; Al-Mousa and Michelangeli, 2012). TBBPA caused activation of caspases (3/7) after the cells were exposed to TBBPA for 12 hours at a 1 to 5 µM concentration range. There was also a transient increase in intracellular [Ca2+] levels and reactive-oxygen-species (ROS) within these neuronal cells. Furthermore, TBBPA also caused rapid depolarization of the mitochondria and cytochrome c release in these neuronal cells (at 10 µM) (Al-Mousa and Michelangeli, 2012). TBBPA was also found to be acutely cytotoxic in primary cultures of rat cerebellar granule cells after 30 minute exposures to 10-50 µM of TBBPA (significant at 25 uM). According to the authors, TBBPA also induced an increase in intracellular Ca2+ concentrations, depolarization of mitochondria, and activation of ROS production (Ziemińska *et al.*, 2012). Mechanistic studies have also shown inhibition of uptake of neurotransmitters dopamine, glutamate and gamma-amino-n-butyric acid (GABA); the IC50 values for TBBPA were 9, 6 and 16 M, respectively (Mariussen and Fonnum, 2003). A more detailed description of this study can be found in the EU RAR 2008 (page 102) but the conclusion is that TBBPA causes inhibition of neurotransmitter uptake and affects membrane potential in rat brain synaptosomes *in vitro.* At low micromolar concentrations, TBBPA increased reactive oxygen species (ROS) formation, extracellular glutamate and intracellular calcium in cerebellar granule cells leading to apoptosis-like nuclear shrinkage, chromatin

condensation, DNA fragmentation and cell death (Reistad *et al.*, 2007). These effects, associated with activation of MAP kinases ERK1/2, may underlie a mode of action of TBBPA. Overall, it has been found that TBBPA is cytotoxic to neuronal cells *in vitro*.

Based on the available data from published studies and confidential study reports, the evaluating MSCA has not made a final assessment and conclusion regarding TBBPA's potential effects on the developing nervous system in mammals. On the one side a range of studies report that no observed effects were obtained whereas other do indicate that such effects might occur. Since TBBPA has been shown to act as a neurotoxic compound *in vitro*, it is furthermore currently difficult to assess whether any potential developmental neurotoxic effects are most likely mediated by thyroid hormone insufficiency or via a more direct neurotoxic effect of TBBPA. The evaluating MSCA has, therefore chosen not to pursue this potential concern for developmental neurotoxicity in mammalian species at this stage. Classification of the potential DNT concern for mammalian species (including humans) is neither a key factor regarding the now identified concern for adverse thyroid effects in amphibians and it is thus nor necessary as supporting evidence for requesting LAGDA

Current evidence on TBBPA and its possible interference with estrogen signaling

Based on a proposal for amendment from a MSCA the concern related to the possible interference with estrogenic signaling is requested to be included in the investigation of the requested LADGA. While in some studies, TBBPA exhibits weak estrogen receptor (ER) activity in vitro (Samuelsen et al. 2001; Kitamura et al. 2005b; Li et al. 2010, Olsen et al. 2003), other studies show equivocal results (Bermudez et al. 2010) and yet other studies found that TBBPA did not exert any estrogenic effects, even at high concentrations (Nishihara et al 2000; Hamers et al. 2006; Dorosh et al. 2010; Riu et al. 2011; Lee et al. 2012). These data suggest that modulation of estradiol receptor signaling is probably not a significant mode of action of TBBPA.

Results of *in vivo* studies investigating effects on estrogen signaling are also mixed. The effects of TBBPA on vitellogenin in fish have been examined in eight studies using four different fish species. Two of the studies showed induced vitellogenin mRNA (Chow *et al.* 2012, Huang *et al.* 2013) whereas no indications for increased production of vitellogenin were observed in five of the studies (measuring vitellogenin protein or studying liver-cell morphology and staining properties) (Christensen *et al.* 2000, De Wit *et al.* 2008, Ronisz *et al.* 2004, Kuiper *et al.* 2007, Song *et al.* 2014, Wang *et al.* 2011). Induction of vitellogenin in two of seven studies raises some concern for estrogenic signaling in fish even though the current data indicate that there might be differences between species. It is also noted that the different studies may have some differences in respect to test design and hence a clear conclusion is difficult to draw.

The (anti)estrogenic potential of TBBPA on birds has been investigated in two long term in vivo studies with Japanese quail (*Coturnix japonica*) and domestic fowl (*Gallus domesticus*). No estrogen-like effects were demonstrated in the two avian studies performed with TBBPA. However, this could be due to the exposure method (yolk injection). Ma *et al.* 2015 did not find any evidence of induction vitellogenin in chicken hepatocytes using an Avian ToxChip polymerse chain reaction (PCR) array and a real-time (RT)-PCR Assay.

Kitamura et al. (2005b) exposed ovariectomized B6C3F1 mice with TBBPA and noted increased uterus weight in all exposed groups suggesting estrogenic activity, however

there was a poor dose-response. In a more recent uterotrophic assay in mice (Ohta et al. 2012) performed in accordance with OECD guideline 440, no estrogenic potential of TBBPA was seen at doses up to 1000 mg/kg/day. Furthermore, the results from the developmental toxicity studies do not indicate estrogenic effects of TBBPA, as no effects on female fertility and no adverse effects on estrous cyclicity, vaginal opening or female reproductive weights and have been observed. However, uterine tumors found in female rats in the NTP two-year cancer study (NTP 2014a) indicate hormonal imbalances of the estrogen axis. These are hypothesized to be caused by inhibition of sulfotransferase, and subsequent decrease in estrogen elimination and increased estrogen levels in serum, leading to increases in uterine tumor formation. There are, however, also other proposed mechanisms of action/MoAs behind the observed uterine tumors.

A newly published study has provided additional information in regards to the mechanisms of action behind the uterine tumors. The North American Flame Retardant Alliance (NAFRA) has funded a targeted 28d rat study investigating this possible mechanism of action (Borghoff et al. 2016). This study investigated the levels of TBBPA and its conjugated forms (TBBPA-GA and TBBPA-S) in liver, plasma and uterus, during and after 28-day exposure. The study results indicated that the sulfation pathway becomes limited with increasing doses of TBBPA, supporting the hypothesis that high doses of TBBPA limit estradiol sulfation, and consequently lead to increased incidence of uterine tumors. Unfortunately no measurements of estradiol levels were performed, neither in plasma nor in relevant tissues like the uterus. The authors state themselves that " *based on the challenges associated with directly measuring estrogens and its metabolites in tissues (e.g. assay sensitivity, specificity and variability) the objective of this study was to determine if conjugation of TBBPA to sulfate would be limited at dose levels associated with development of... uterine tumors in rats...*". However, results of actual estrogen levels in plasma and tissue would have been very informative in regards to assessing the proposed mechanism of action.

Hamers et al. (2006) found, however, TBBPA to be a very potent inhibitor of 17β-estradiol sulfotransferase (E2SULT) and suggested that observed estrogenic activity of TBBPA might be explained by its inhibiting effect on sulfotransferase rather than a direct effect on ER activity.

Why new information is needed

Based on the information described above, the evaluating MSCA has identified a concern for the possible endocrine disruptive properties of the registered substance in vertebrate non-mammalian wildlife species as there is a concern for TBBPA affecting thyroid hormone signalling. As already mentioned above, TBBPA decreases serum T4 levels in adult rats suggesting that TBBPA might adversely affect brain development after perinatal exposure. However, since TBBPA has also been observed to be able to induce cytotoxicity in various neuronal cell types, further testing in rodents does not seem to be the most appropriate way forward at this stage since it is questionable whether further studies in rats can help to distinguish between whether any observed changes on brain development are mediated by thyroid hormone interference or by a non-endocrine related type of effect (e.g. neurotoxic effect not caused by hormone interference) of TBBPA exposure on the developing brain. However, effects of TBBPA on thyroid signalling observed in fish and amphibians are also demonstrated in a number of available studies. The concern for adverse effects of TBBPA relevant to thyroid signalling is therefore requested to be investigated in amphibians, also because of the existence of OECD test guidelines suited for such investigations.

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In order to identify whether the registered substance is an endocrine disruptive substance, the WHO definition *"An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations"* (WHO/International Programme on Chemical Safety 2002) as interpreted by the EU Expert group (Munn and Gourmenou, 2013) *"that the elements for identification of an endocrine disrupter [are] demonstration of an adverse effect for which there [is] convincing evidence of a biologically plausible causal link to an endocrine disrupting mode of action and for which disruption of the endocrine system [is] not a secondary consequence of other non endocrine-mediated systemic toxicity"*, will be applied.

As the available data do not include recognized adverse effects linked to disruption of the thyroid signalling in amphibians, a Larval Amphibian Growth and Development Assay (LAGDA, OECD TG 241) is requested in order to investigate possible adverse effects caused by the TH antagonism observed in the above referred amphibian studies. The results of the test will be used to evaluate whether the registered substance meets the above mentioned criteria for endocrine disrupters and the criteria in REACH article 57 (f). *"substances — such as those having endocrine disrupting properties [...] for which there is by providing scientific evidence of probable serious effects to human health or the environment which give rise to an equivalent level of concern to those of other substances listed in points (a) to (e)[4] and which are identified on a case-by-case basis in accordance with the procedure set out in Article 59".* It is noted in this context also that presently TBBPA has a harmonized classification according to the CLP regulation as Aquatic Acute 1 and Aquatic Chronic 1 and that the substance is not readily biodegradable, has a long half-life in the environment and also has been concluded to have a significant BCF in fish of 1234 based on C14 measurements (c.f. EU RAR 2008, c.f. also the section below on Endpoint 5 (PBT properties)).

TBBPA and its possible interference with estrogen signaling

There is an unclarified concern identified in the course of the substance evaluation, which was highlighted based on a PfA from a MSCA, in relation to the evidence of possible estrogenic effect probably induced by TBBPA inhibiting E2 sulfotransferase.

Considerations on the test method and testing strategy

The LAGDA serves as a higher tier test which is placed at level 4 of the OECD ED conceptual framework (OECD 2012) with an amphibian species for providing test data on thyroidal mode of action and data on its plausible link to related serious (adverse) effects on development.

LAGDA is requested and not the potential alternative test the Amphibian Metamorphosis Assay (AMA) (OECD TG 231), because several non-guideline mechanistic amphibian metamorphis tests have already been performed, as summarised above, indicating a thyroid MoA hence the concerns for thyroidal effects in amphibian species supported by certain available fish and rat studies (cf. above) are sufficiently significant to warrant a LAGDA, which is providing more definitive conclusions as regards endocrine disruption in amphibian species than the AMA, which is only recognized as providing evidence for a thyroidal Mode of Action (MoA). This is also reflected in the fact that LAGDA is placed at level 4 of the OECD ED CF whereas AMA is placed at level 3.

[4] i.e. CMRs and vPvBs/PBTs

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Based on your comments, in order to clarify whether internal T3 levels affect the responses of TBBPA as indicated by several studies (Veldhoen et al., 2006, Zhang et al., 2014; Zhang et al., 2015), the requested LAGDA should include T3 concentration analyses on 5 animals per replicate at Interim Sampling (Larval sampling) and on at least 5 animals per replicate at test termination (Juvenile sampling). T3 analysis should be performed on plasma. Plasma sampling should be done in NF stage 62 larvae at interim sampling if possible. In addition, at interim sampling, analysis should be performed on homogenate of the lower torso posterior to the forelimbs which is otherwise discarded (no further animals would be needed). You should seek advice on T3 quantification in OECD (2006), from the LAGDA contract laboratory and in relevant literature (e.g. Krain & Denver, 2004) to include the most sensitive T3 quantification method available. In order to address your comments regarding positive controls and also the comments regarding partial agonist action, a positive control exposed to 1 µg/L T4 for agonistic thyroid activity shall be included in a minimum of 4 replicates and a positive control exposed to 50 mg/L 6-n-propyl-2-thiouracil (PTU) for antagonistic thyroid activity shall be included in a minimum of 4 replicates. As you highlighted, iodine, fluoride, and perchlorate can affect the thyroid pathway in amphibians. Therefore, during the exposure period, the concentrations of iodine, fluoride and perchlorate as for TBBPA shall be determined at appropriate intervals, preferably every week for at least one replicate in each treatment group, rotating between replicates of the same treatment group every week.

Estrogenic effects have been observed in some *in vitro* and some *in vivo* studies whereas other studies did not show such activity/effects. Furthermore, uterine tumours found in female rats in the NTP two-year cancer study indicate that TBBPA exposure may result in hormonal imbalances of the estrogen axis of rats. The estrogenic effect is might when observed be caused by inhibition of sulfotransferase activity by TBBPA and subsequent decrease in estrogen elimination and increased estrogen levels in serum. This may in the above mentioned cancer studies on rats have led to the observed increases in uterine tumour formation.

The OECD TG 241 includes an optional part with plasma VTG measurement. As there are indications of estrogenic effects of TBBPA as summarised above, plasma VTG shall be measured in the requested LAGDA. The measurements shall be performed as described in the guideline. It is noted that the concern regarding estrogenic effects of TBBPA is now only addressed by addition of an estrogen sensitive parameter to the already requested LAGDA, and not with a request of further testing on laboratory animals. This will be considered depending on the results for the now requested information and the above mentioned already initiated American rat studies have been reported and evaluated.

The evaluating MSCA must have access to the robust study summaries as well as the full study report including all relevant details of the study, ensuring that a clear conclusion regarding the result of the study can be drawn by the evaluating MSCA. The reason for requesting the full study report is that its accessibility to the evaluating MSCA is most probably needed in order to evaluate all study details relevant for the result because such details are based on general experience on higher tier test not always available in robust study summaries only.

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Alternative approaches and Proportionality of the request

If AMA was requested first and, as most likely is positive, this result would lead to a request for further animal testing with LAGDA. Hence requesting AMA followed by LAGDA would employ more animals for testing in total, than by requesting the confirmatory LAGDA now.

Since both endocrine mode of action and the occurrence of adverse effects are prerequisites for identification of an endocrine disruptors, data from the LAGDA can be used directly to identify the substance as an endocrine disruptor, whereas positive results from the AMA would need to be supported with other data, in this case most probably with a follow up request for testing in the LAGDA. ECHA also notes that there is currently no experimental study method available that does not employ vertebrate animals that could generate the necessary information. Hence requesting LAGDA is the most suitable way of targeting the concerns and at the same time taking the laboratory animal welfare (3 R principles) and testing cost for you into account.

The request for LAGDA is suitable and necessary to obtain information that will allow to clarify whether TBBPA can disrupt normal thyroid signalling *in vivo* in a vertebrate species. More explicitly, there is no equally suitable alternative way available of obtaining such information. If the data, once obtained, confirms that the substance has endocrine disruptive properties linked to adverse effects it will allow authorities to consider further regulatory risk management in form of e.g. additional regulation through REACH article 57 (f), i.e. nomination for Candidate Listing as the initial step in the Authorization scheme of the REACH Regulation.

Consideration of your comments

You did not consider the immediate requirement of a Level 4 test according to the OECD Conceptual Framework on Endocrine Disruptor Testing and Assessment as proportionate. You argued that OECD 241 LAGDA as a higher tier (Level 4) test requires knowledge from previous research and is normally carried out in the light of previous preliminary experimental work, on the basis of which the correct protocol details are established. You believe that such preliminary experimental information is not currently available as you argued that the data cited by the evaluating MSCA and data from other published literature lack crucial elements of the test details that are important for the judgment of the reliability, validity and relevance of the studies (e.g. pH, iodine, fluoride, perchlorate content of the medium in the case of *in vitro* studies, measured concentrations of the substance in the test system during the studies, consideration of cytotoxicity, positive controls, historical control values, and organisms husbandry conditions *in vivo* studies).

ECHA highlights that the OECD Conceptual Framework is not a testing strategy and it is described in Note 1 that "Entering at all levels and exiting at all levels is possible and depends upon the nature of existing information". ECHA considers the WoE of TBBPA thyroid interference as adequate for requiring a Level 4 test that could inform about mechanistic as well as adverse effects of TBBPA on amphibian development. ECHA agrees that some of the available studies have limitations/shortcomings compared to OECD and USEPA standard test methods and that this should be taken into account when evaluating the results, which has also been the case during evaluation of the TBBPA literature by the evaluating MSCA. Unfortunately no validated guideline studies with endocrine relevant endpoints have yet been performed in wildlife species such as in fish or amphibian species. As TBBPA exposure concentrations in most of the aquatic

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toxicity studies were not measured analytically the results are used to inform about mechanism/mode of action of TBBPA not to define effect levels (e.g. for quantitative risk assessment). Lack of analysis of iodine, fluoride, perchlorate content of the medium is noted but not a caveat that disqualifies data because it is not a mandatory requirement in neither TG 231 and 241, but only recommended. Control groups (i.e. negative controls) are included in all the evaluated studies and prevent effects of these anions to be mistaken as TBBPA related effects. ECHA agrees that it could be important to measure iodine, fluoride, and perchlorate levels in exposure water why based on your comments it has been included in the study design for the requested LAGDA. Positive controls are also not required in TG 231 and 241. However based on this comment from you positive controls are now also included as mandatory in the request for the LAGDA on TBBPA. Inclusion of control groups minimises e.g. theoretically stress effects suggested by you to be being mistaken as effects of TBBPA exposure. Regarding historical range of values for the endpoints reported it is quite difficult to obtain such data when test methods/endpoints included in test methods are relatively newly adopted. But control groups should prevent mistaking effects from bad husbandry with exposure effects. Please also note that the results reported are not used as definitive evidence for concluding that TBBPA causes thyroidal linked adverse effects in amphibians but only for concluding that there is concern for such effects and hence reason to request the LAGDA. ECHA agrees that solvent concentrations have been higher than allowed according to the current standard TGs (AMA and LAGDA) in several of the available and reported wild amphibian species studies, but also that they have included a solvent control which makes it less likely to obtain false positive effects of the TBBPA exposure.

You argued that available literature has not adequately investigated the hypothesis provided in the original draft decision (e.g., partial agonist with differential responses based on T3 levels) and should be clarified before proceeding, if necessary, to a Larval Amphibian Growth and Development Assay (LAGDA). You argued that an OECD 231 Guideline Amphibian Metamorphosis Assay (AMA) study could be conducted to determine the validity of this hypothesis, because the study would be conducted over the natural increase in endogenous T3 during development. Adding a high dose of T3 exogenously potentially creates an environment that is not natural for the organism according to your comment.

ECHA highlights that T3 analysis is, as in many of the reported studies, not a part of the mandatory endpoints in neither AMA nor LAGDA and therefore possible effects of TBBPA cannot be correlated to T3 in any of these tests unless T3 concentration analysis is included as an endpoint. Based on your comments T3 analysis is now included in the study design. ECHA disagrees that the current data are "inconsistent". A likely explanation for the dual effects of TBBPA, showing both agonistic as well as T3 antagonistic effects in the in *vitro data* on TR agonism and antagonism, is that TBBPA works as a partial agonist, which is one that can display both agonistic and antagonistic effects. When both a full agonist (T3) and partial agonist are present, the partial agonist actually acts as a competitive antagonist, competing with the full agonist for binding to the receptor, decreasing the response observed with the full agonist alone. The argument that the literature has not adequately investigated the hypothesis regarding partial agonism does not hold, as there is no specific test guideline for investigating a potential partial agonist. You make the argument based on the data from the agonist and antagonist studies, and such studies have been reported numerously in the literature. Thus the hypothesis of TBBPA being a partial agonist is supported by the literature. ECHA is of the opinion that based on WoE of all available studies, including

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both *in vivo* studies on amphibians and in vitro studies, that there is sufficient causes for concern to request a LAGDA as now specified in this decision. ECHA does not agree with you that more mechanistic *in vitro* studies are warranted before such a study is requested. LAGDA is relevant to request at this stage to reach a regulatory decision on whether based on its results it can be concluded that the substance can disrupt the endocrine system in an intact animal wildlife species.

You highlighted that OECD 241 (July 2015) is a new and complex guideline and that there is limited experience and capacity in respect to the conduct of the test and lack of historical control values concluding that this all together suggests that this test should not be required in a regulatory context. You argued that gene expression test data and *in vitro* data regarding Key events of the Thyroid AOP should not be used because the meaning of such data is currently unknown or may not be relevant *in vivo*, respectively. Hence you did not agree that such data could be used to support the requested LAGDA. You highlighted that several Mechanistic Initiating Events (MIE) are possible when dealing with thyroid related effects and argued that the evaluating MSCA had not identified which MIE was relevant for the effects observed in the studies. Based on the concerns described above you suggested performing instead an AMA test according to OECD 231 to address the concern of the evaluating MSCA on possible Thyroid effects in wildlife.

ECHA highlights that the LAGDA is an adopted OECD TG and therefore recognized as a validated test method to request when warranted for regulatory purposes. It is important to emphasize that the OECD ED Testing and Assessment Conceptual Framework (CF) is not a testing strategy and it is described in Note 1 of the ED CF that: "Entering at all levels and exiting at all levels is possible and depends upon the nature of existing information and needs for testing and assessment".

You highlighted that none of the studies cited complied with Good Laboratory Practices (GLP) thus it cannot be concluded that these studies are of sufficient quality due to details which are likely not available or were not considered (e.g., calibration of balances) for the cited studies.

ECHA acknowledges that it is well known that the academic studies published in the scientific literature normally do not follow GLP but that such studies nevertheless may contain reliable information especially when several studies are evaluated together based on weight of evidence. In this case, there is a lot of important information in these studies which has been used in the performed WoE analysis as summarised in this decision. ECHA also stresses that GLP in itself is not providing any guarantee in respect to whether scientifically relevant endpoints are investigated. Hence no revisions were made based on these comments from you.

You did not agree with the identification of possible mammalian DNT effects as described in the original draft decision. You highlighted that several other authorities have dismissed this concern. You also argued that some of the studies included in the WoE are not suitable for hazard identification.

ECHA has revised the statement of reasons regarding DNT in mammalian species but has not expanded the text much as the potential concern for this endpoint has not been concluded yet. This potential concern is not a key factor regarding the identified concern for adverse thyroid effects in amphibians, only the observed T4 serum levels in rats has been noted as only one of the elements of supporting evidence for requesting a LAGDA now.

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ECHA is of the opinion that based on WoE related to all available studies including *in vivo* studies on mammalian but in particular non-mammalian vertebrates and *in vitro* studies there is sufficient causes for concern to request a LAGDA as now specified in the revised DD.

In a comment to a PfA from a MSCA you disagreed with the proposal to include a mandatory request for measuring plasma VTG in the requested LAGDA as he claimed that studies in fish and birds have already investigated whether TBBPA alters vitellogenin (Vtg). According to your comment these studies clearly show that TBBPA does not alter Vtg levels, except at concentrations where mortality also occurs (Chow *et al.* 2012). Therefore, you concluded that measurement of Vtg in an amphibian study is not warranted. You further argued that the use of a fish model is more common for Vtg measurement and analysis. You further felt that the mandatory endpoints (e.g. gonadal duct histopathology, genotypic/phenotypic sex ratio) in the OECD 241 protocol already allow for assessment of chemicals with potential mixed modes of action. For instance, oviduct formation in amphibians is highly correlated to circulating estradiol levels and thus serves as an excellent indicator of a chemicals estrogenic activity (OECD, 2015). Therefore, the inclusion of Vtg into the test battery would in your view not enhance the TBBPA, especially in light of the existing data which report a lack Vtg induction following TBBPA exposure.

ECHA agrees that targeted fish testing (OECD TG 229, 230, or 234) is more common for Vtg measurement and analysis. However, the inclusion of VtG measurements in the LAGDA is possible and could be used as an alternative to further testing in fish and that this is particular relevant when the LAGDA is anyways requested considering that the concern for estrogenic effects in fish is only moderate and in the light of animal welfare considerations. As explained above the two positive fish studies raise some concern for effects estronic signaling. Furthermore, the inclusion of VtG measurements, in the requested LAGDA, does not include additional use of laboratory animals. ECHA does not agree with the argument that TBBPA did not alter Vtg levels, except at concentrations where mortality also occurs. For zebrafish larvae a NOEC of 2.64 mg/L and a LOEC of 3.95 mg/L for induction of Vtg were reported. As observed from the concentration-response curve for larvae mortality in Chow et al., 2012 (Figure 2E), no significant mortality was registered below 3.95 mg/L

In a comment to a PfA by a MSCA you argued that the mandatory endpoints (e.g. thyroid histopathology) in the OECD 241 protocol already allow for assessment of substances with specific thyroid activity and that T3 is not a mandatory endpoint nor is it listed as an optional endpoint. You further argued that the inclusion of T3 into the test battery will not enhance the TBBPA ecological risk assessment as the study focuses on key apical endpoints (i.e. survival, growth and reproduction). You further stated that adequate background data and method evaluation data, including the circadian variations, are mostly not available and it will be difficult to evaluate the results.

ECHA reminds you that the T3 measurements was included in the decision based on your comments on the draft decision criticising published data on thyroidal effects in amphibian species as further elaborated above and has therefore kept this testing requirement.

Conclusion

Therefore, based on the substance evaluation and pursuant to Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following

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study using the registered substance subject to this decision: The Larval Amphibian Growth and Development Assay (LAGDA); test method: OECD 241.

ENDPOINT 2: Dissociation Constants in water

The Concern(s) Identified

Based on a study submitted in the Registration dossier TBBPA is not readily biodegradable ([redacted]). Transformation products include mono- and bismethyl ether TBBPA which both have potential PBT properties according to QSAR predictions (see Endpoint 5 and 6 for further information).

Why new information is needed and test method/testing strategy considerations.

A valid water solubility test value is not available on the monomethyl ether TBBPA and uncertainties remain regarding the actual water solubility (see Endpoint 3). Due to the potential deprotonation of the monomethyl ether TBBPA, its pKa shall be determined to be able to take into account the degree of ionization of the substance when afterwards first water solubility and then the degradation rate is determined. Furthermore, the degree of ionization (in the environmentally relevant pH range 5 to 9) may significantly influence the bioconcentration factor in fish (Trapp *et al.*, 2010).

The evaluating MSCA must have access to the robust study summaries as well as the full study report including all relevant details of the study, ensuring that a clear conclusion regarding the result of the study can be drawn by the evaluating MSCA. The reason for requesting the full study report is that its accessibility to the evaluating MSCA is most probably needed in order to evaluate all study details relevant for the result because such details are based on general experience on higher tier test not always available in robust study summaries only.

Alternative approaches and proportionality of the request

In the OECD 105 TG for water solubility it is stated: "Before determining water solubility, it is useful to have some preliminary information on the substance, like structural formula, vapour pressure, dissociation constant [...]"

Also, in ECHAs guidance on IR and CSA it is stated that "For ionising substances, the pH-dependence of the water solubility should be known."

Monomethyl ether TBBPA has an acidic proton and dissociation is expected at environmental relevant pH.

Alternatively, some QSAR methods exist for predicting the pKa (SPARC, ACD/Labs), however, most of these QSAR models require license. Use of QSAR predicted pKa-values include some degree of uncertainty.

Consideration of your comments

This test has been included based on your recommendation provided in your comments to the original draft decision.

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Conclusion

Therefore, based on the substance evaluation and pursuant to Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following study **using the transformation product monomethyl ether TBBPA**: A Dissociation Constants test using the OECD 112 (Dissociation Constants in Water).

ENDPOINT 3 Water solubility

The Concern(s) Identified

TBBPA is not readily biodegradable ([redacted]). Transformation products include monomethyl and bismethyl ether TBBPA which both have potential PBT properties according to QSAR predictions (see Endpoint 5 and 6 for further information).

No test data for water solubility of monomethyl ether or bismethyl ether TBBPA are available in the registration dossier or have been identified by the evaluating MSCA. As monomethyl ether TBBPA has been shown to be a degradation product of the registered substance in water (Peng *et al.,* 2014) and water covered soil (Sun *et al.*, 2014), and also has been found in the aquatic environment (in SPM; 2.3-4.5 µg/kg dw, and fish muscle tissue; up to 1.84 µg/kg ww) ([redacted]), reliable data on the water solubility of monomethyl ether is relevant for assessment of its persistency in surface water.

QSAR estimated water solubility values have been obtained from EPIWEB 4.1 of the US Environmental Protection Agency using the logKow and the fragments method, respectively.

Since no test data are available, logKow is also estimated. The QSAR predictions (using VEGA 1.1.1[5] and EPI 4.1[6]) yield a logKow value of 5.47-8.23 for monomethyl ether TBBPA.

The water solubility estimate for monomethyl ether TBBPA based on logKow (WSKOW v1.42) using a logKow of 8.23 yields a water solubility at 25°C of 0.11 µg/L. Using a logKow of 5.47 yields a water solubility at 25°C of 24.53 µg/L. Using the fragments method yields a water solubility of 24.14 µg/L. It is noted that the latter value is approximately equal to the water solubility when estimated by the logKow based water solubility estimation method when based on the lowest logKow value estimate. The water solubility based on the high logKow estimate is two orders of magnitude lower than the water solubility based on the fragment method. This may indicate that the water solubility based on the low logKOWWIN based value might be more reliable than the one based on the high logKow value and also that the low logKow value may be the most reliable logKow value. However, it is from the differences between these estimations clear that the actual water solubility value of monomethyl ether TBBPA is uncertain. What can be concluded currently is that based on these data, a water solubility value of 0.11-24 µg/L seems likely even though the higher value is most probably the most reliable estimate. This leaves significant uncertainty with regard to the exact water solubility of monomethyl ether TBBPA across the range of 1-10 µg/L which is normally employed in the kinetic part of simulation degradation testing in TG

[5] http://www.vega-qsar.eu/
[6] http://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface

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309 which is one of the simulation degradation tests to use when exploring further the environmental persistency (cf. above).

For the other O-methylated transformation product of TBBPA, bismethyl ether TBBPA, logKow QSAR predictions (using VEGA 1.1.1 and EPI 4.1) varied between 5.69 and 8.84. The water solubility estimate using a logKow of 8.84 (VEGA v. 1.1.1 LogKowWIN) yields a water solubility at 25°C of 0.007 µg/L. Using a logKow of 5.69 (VEGA v. 1.1.1 MlogP) yields a water solubility at 25°C of 3.39 µg/L. Using the fragments method yields a water solubility estimate of 0.41 µg/L. It is noted that the water solubility range between 7 nanogram and 4 microgram, showing a consistent very high degree of hydrophobicity but also very high uncertainty of three orders of magnitude. Based on the predicted water solubility value and logKow values it is considered that it may not practically be possible to test the environmental persistency of this transformation product in surface water due to low solubility. It is noted in the updated registration dossier that bismethyl ether TBBPA has a solubility in water of 63 ng/L. The study report has not been available to the evaluating MSCA but based on QSAR estimated values this value seems plausible even though it must be concluded, based on available information, that the water solubility is uncertain but very low.

Why new information is needed

A valid water solubility test data value is not available on the monomethyl ether TBBPA and uncertainties remain regarding the actual water solubility as QSAR estimated water solubility values differs about two orders of magnitude. As water solubility is important for assessing the relevant environmental compartment of concern for further degradation testing and in respect to the feasibility of conducting a surface water simulation degradation study on monomethyl ether TBBPA, a more accurate value on water solubility than those provided by use of QSAR based estimations, is necessary.

Considerations on the test method and testing strategy

Due to its hydrophobicity, monomethyl ether TBBPA (logKow 5.47-8.23 based on EPIWIN v.4.1 and VEGA 1.1.1) is expected to adsorb to test vessels. Therefore, a full mass balance of the following samples shall be analysed:

- the clear aqueous phase,
- the remaining test solution in the sample container,
- a solution of any test substance (by using a suitable solvent) remaining in the sample container after disposal of the original test solution.

Based on your comments the water solubility test shall be performed at 12 °C. Prior to the solubility test the dissociation constant in water should be determined (cf. Endpoint 2 above). The best suited simulation study to investigate the biodegradation of monomethyl ether TBBPA will depend on the water solubility and other environmental fate properties. You shall justify if, depending of the particular column used, the water solubility measured only refers to the unionized part of the substance at the particular pH value of the test and how account of this should be taken in respect to the follow up simulation degradation testing.

The evaluating MSCA must have access to not only the robust study summaries but also the full water solubility study report including all relevant details of the study, ensuring that a clear conclusion regarding the result of the study can be drawn by the evaluating MSCA. The reason for requesting the full study report is that its accessibility to the

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evaluating MSCA is most probably needed in order to evaluate all study details relevant for the result because such details are based on experience not always available in robust study summaries only.

Alternative approaches and Proportionality of the request

The request for a water solubility test is appropriate and necessary to obtain accurate information on solubility in water that will allow further assessment of the persistence and, if needed the bioaccumulation potential and chronic toxicity to aquatic organisms, of monomethyl ether TBBPA. More explicitly, there is no equally suitable alternative way available of obtaining this information. As explained above, the QSAR predictions for this endpoint are uncertain.

Consideration of your comments

You argued that since the result of the water solubility test will be the basis for the decision on the appropriate degradation study to be performed (either OECD 309 (if water solubility is > 1µ/L) or OECD 308), the solubility should be tested using the same temperature and test medium as recommended in the guideline 309. Furthermore, you argued that due to the potential deprotonation of the monomethyl ether TBBPA, its pKa should be determined prior to the performance of the solubility study in order to identify the form that will need to be analyzed during the test.

ECHA agrees that the water solubility test and the simulation degradation test should be performed at the same temperature as the water solubility of organic substances is temperature dependent. The solubility in water is not expected to be significantly affected by the OECD TG 309 test medium (surface water) as this does not have a high salt concentration. Based on your comments and these considerations, ECHA requests you to perform the water solubility test also at 12 °C. Prior to the solubility test the dissociation constant in water should be determined (c.f. above point 2).

In a comment to a PfA from a MSCA you argued that the water solubility should not only be tested in pure water. You suggested that if the water solubility in pure water is in the range of 1ug/L, the solubility in the test medium should be also determined in order to be able to make a decision on a feasible and appropriate approach for the biodegradation testing.

ECHA has not included this in the requested test design, however, you are free to perform a water solubility test in the test medium if you think this is warranted. It should be noted that if the test medium contains a high amount of SPM, it would be expected that monomethyl ether due to its hydrophobicity will adsorb to the present SPM. As this would be removed before analysis of the concentration of freely dissolved test substance in water this could underestimate the "real" water solubility also depending on whether steady state between the organic phase and the water phase is reached or not. Should you use the result of a solubility test in regards to the requested surface water degradation simulation test medium (surface water with the above specified SPM concentration(s)) as a reason why it is not considered technically feasible to test the persistency of monomethyl ether TBBPA in surface water the issue regarding adsorption of monomethyl ether TBBPA to SPM in both the test system and in surface water should be convincingly addressed.

Therefore, based on the substance evaluation and pursuant to Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following

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study **using the transformation product monomethyl ether TBBPA**: Water solubility test (Column elution method, EU A.6/OECD 105) with expansion of the test design to cover a full mass balance of the test substance as described above. The test shall be performed at a temperature of 12°C.

ENDPOINT 4: Partition Coefficient (1-Octanol/Water)

The Concern(s) Identified

TBBPA is not readily biodegradable ([redacted]). Transformation products include monomethyl ether TBBPA which has potential PBT properties according to QSAR predictions (see Endpoint 5 and 6 for further information).

Why new information is needed and test method/testing strategy considerations.

To evaluate the persistency of monomethyl ether a simulation degradation study in surface water with a SPM content of approximately 5 and 30 mg dw/L is requested. Due to the potential adsorption of the monomethyl ether TBBPA to organic material, its partition coefficient (log Kow) shall be determined to take into account the degree of adsorption to (suspended) sediment.

No measured logKow is available for monomethyl TBBPA. QSAR predictions for logKow based on different models predicts a high lipophilicity/hydrophobicity; 5.47 (Vega MlogP) 6.97 (AlogP), 7.76 (EPI KOWWIN), and 8.23 (Vega LogKowWIN). The QSAR predictions estimate a logKow value of 5.47-8.23 for monomethyl ether TBBPA. The OECD TGD 107 covers a logKow range of -2 to 4 whereas the OECD 117 covers a logKow range of 0 to 6. The OECD 123 ("Slow stirring method") can measure log Kow values up to 8.2. Therefore OECD 123 is the most appropriate test to select for the direct determination of log Kow for a highly hydrophobic substance like monomethyl ether TBBPA.

In respect to the possible ionization of monomethyl ether TBBPA: if relevant, you should justify if the analytical method employed in the OECD 123 test measures the log Kow of the unionized fraction of monomethyl ether TBBPA at the particular pH value in the test.

The evaluating MSCA must have access to the robust study summaries as well as the full study report including all relevant details of the study, ensuring that a clear conclusion regarding the result of the study can be drawn by the evaluating MSCA.

Alternative approaches and proportionality of the request

In the OECD TGD 309 paragraph 10 it is stated that *"If the test is carried out as a "suspended sediment test" the following information should also be available: [...] - n-octanol/water partition coefficient [OECD 107, 117]".* As the test will be performed with a SPM content of ~5 mg dw SPM /L and ~30 mg dw SPM/L it is relevant to know the adsorption potential. Furthermore, should it be technically unfeasible to perform the degradation simulation test in surface water due to analytical limitations and the low water solubility, a simulation degradation test in sediment is then requested. In the OECD TG 308 paragraph 8 it is also stated that the octanol/water partition coefficient should preferably be available before carrying out this test.

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Consideration of your comments

This test has been included based on a proposal for amendment from a MSCA. You did not comment on this proposal.

Conclusion

Therefore, based on the substance evaluation and pursuant to Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following study **using the transformation product monomethyl ether TBBPA**: Partition Coefficient (1-Octanol/Water): OECD 123: Slow-Stirring Method.

ENDPOINT 5 PBT properties: Environmental fate of the transformation product monomethyl ether TBBPA (no CAS available): Simulation degradation testing

The Concern(s) Identified
The evaluating MSCA has identified a concern for the environmental fate of the methylated transformation product of TBBPA: monomethyl ether TBBPA (no CAS available).

Available studies from the open literature show that TBBPA can be converted to two distinct methylated transformation products, monomethyl ether TBBPA and bismethyl ether TBBPA (CAS 37853-61-5) in amounts significant to PBT assessment. As most available studies cover both degradation metabolites, the findings are described together here as it gives the most coherent picture of the degradation pathway(s) of TBBPA, including the formation of monomethyl- and of bismethyl ether TBBPA, in different environmental compartments.

O-methylation of TBBPA to its monomethyl and bismethyl ether derivatives has been shown to occur in natural sediments (George and Häggblom, 2008). Two natural sediments, Kearny Marsh (New Jersey, USA) and Kymijoki River (Finland) (sampled and stored at 4°C, added to vessels as slurry) were spiked with a TBBPA concentration of 10 μM. After 80 days, 25% of the original TBBPA was transformed to monomethyl ether TBBPA and 35% of the original TBBPA was transformed to bismethyl ether TBBPA in sediment from Kearny Marsh. Further, up to 10% of the heterotrophs in this sediment were shown to be capable of O-methylation using a most probably number (MPN) assay.

In sediment from the Kymijoki River approx. 5 % of TBBPA was transformed to monomethyl ether and 5% of TBBPA was transformed to bismethyl ether. Sterile sediment controls showed no activity. The authors showed that the observed O-methylation of TBBPA was microbially induced, using two different bacteria species. Both *Mycobacterium fortuitum* CG-2 and *Mycobacterium chlorophenolicum* PCP-1 were able to transform TBBPA to its methylated derivatives, with *Mycobacterium fortuitum* CG-2 having a faster rate of transformation and a near complete (almost 100%) transformation of TBBPA to its bismethylated ether after 10 days. After 6 days approx. 20% and 10% of the original TBBPA was transformed to TBBPA monomethyl and bismethyl ether, respectively, by *Mycobacterium chlorophenolicum* PCP-1 and this level was constant until the end of the experiment at day 14. This study supports the hypothesis that monomethyl and bismethyl ether TBBPA are products of microbial O-methylation of TBBPA and this transformation is likely in the natural environment.

Sun *et al.* (2014) confirmed the transformation of TBBPA to its methylated ether

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derivatives (mono- and bismethyl ether TBBPA) to occur under aerobic conditions in flooded soil and soil/plant systems. In reed planted soil, approx. 10% of the originally added TBBPA was transformed to its methylated transformation products after 10 days. In rice and unplanted soil approx. 8% of the originally added TBBPA was transformed to its methylated transformation products after 35 days. The levels of methylated metabolites were constant for all three systems until the end of the experiment at day 66, indicating high persistency of the methylated transformation products. The initial concentration of TBBPA in the soil was 5 mg/kg dw. The individual amounts (in %) of monomethyl and bismethyl ether TBBPA formed are not stated.

The transformation of TBBPA to its O-methylated transformation products has also been shown in an agricultural field in Switzerland (Li *et al.*, 2015), where a continuous formation of methylated transformation products was formed, continuously rising to a total of approx. 12% of the initial amount of TBBPA after 143 days (individual amounts (in %) of monomethyl and bismethyl ether TBBPA formed not stated). No steady state was observed, but the continuous rise in methylated derivatives suggests persistence of the metabolites. At the end of incubation, eight extractable metabolites were detected, including TBBPA methyl ethers, single-ring bromophenols, and their methyl ethers.

Biotransformation of TBBPA by freshwater green microalgae was the dominant process in TBBPA removal in freshwater with transformation via sulfation, glucosylation, O-methylation and debromination (Peng *et al.*, 2014). Amongst four other transformation products, TBBPA monomethyl ether was formed by one of the six algal species.

In conclusion, available studies from the open literature currently not assessed but briefly described in the registration dossier of TBBPA show that O-methylating of TBBPA is an important degradation pathway under aerobic environmental conditions and is likely to occur in water, sediment and soil - forming 10-60% total methylated transformation products from the originally present amount of TBBPA. From the current literature it is plausible that up to 10% of naturally occurring bacteria in the environment are able to transform TBBPA to its O-methylated transformation products under aerobic conditions. The available studies indicate that the methylated transformation products of TBBPA, including monomethyl and bismethyl ether TBBPA, may be persistent and are formed at significant levels far exceeding the 0.1% trigger relevant for PBT assessment of transformation products.

Why new information is needed

The evaluating MSCA has identified a concern for the possible PBT properties of the transformation product of the registered substance; monomethyl ether TBBPA.

Persistency
No test data on the potential biodegradation of monomethyl ether TBBPA has been found.

However, data from soil and sediment studies indicate persistency in the environment (George and Häggblom, 2008; Sun *et al.*, 2014; Li *et al.*, 2015). As test results are not available, QSAR predictions using BIOWIN 4.10 (US-EPA QSAR package EPIWIN) and the Danish QSAR database[7] are presented below.

[7] http://qsardb.food.dtu.dk/database/index.html

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Biowin1 (linear model) Probability of Rapid Biodegradation: 0.10
Biowin2 (non-linear model) Probability of Rapid Biodegradation: 0.00
Biowin3 Expert Survey Ultimate Biodegradation: 1.21
Biowin4 Expert Survey Primary Biodeg: 2.39
Biowin5 (MITI linear model) Prob. Biodeg: 0.08
Biowin6 (MITI non-linear model) Biodegradation Probability: 0.01
Danish QSAR database: NRB

Note that BIOWIN 1 & 2 score of < 0.5: predicted not rapidly biodegradable. BIOWIN 5 & 6 < 0.5: predicted Not readily Biodegradable (NRB, according to MITI 1 training set), BIOWIN 3 and 4 gives the ultimate and primary timeframe for degradation (1 year, 2 month, 3 weeks, 4 days, 5 hours). The Danish MCase biodeg. prediction directly indicated whether RB or NRB (based on mainly MITI data training set).

Biowin sub-models 1 and 2 depict that the substances will not degrade rapidly, supported by the Biowin 5 and 6 predictions and the DK QSAR DB. The persistency screening algorithm: BIOWIN2 and/or BIOWIN 6 < 0.5; BIOWIN 3 < 2.2 (2.7), is furthermore fulfilled (ECHA 2014). The molecular weight of monomethyl ether TBBPA (557.9) is within the molecular weight domain of all the models, for which the lowest molecular weight values vary between 30.02 – 53.06 and the maximum values vary between 697.65 – 959.2.

Thus, monomethyl ether TBBPA meets the screening criteria for being P/vP.

Bioaccumulation potential

No test data or field investigations of bioaccumulation or food-chain transfer of this transformation product are available. The QSAR predictions from EPIWEB 4.1 and VEGA 1.1.1 (as described in Endpoint 4) yields a logKow value of 5.47-8.23. Thus, the estimated logKow values are above the screening trigger value of logKow > 4.5, which is the triggering value for bioaccumulation potential (ECHA, 2014). This is supported by the observed BCF and BAF values[8] predicted by BCFWINNT as presented below:

BCF_{fish} = 1888-3321 L/kg ww
BCF Arnot-Gobas method (upper trophic) inc. biotrans. = 831-4387
BCF Arnot-Gobas method (upper trophic) exc. biotrans. = 2577-151000
BAF Arnot-Gobas method (upper trophic) inc. biotrans. =14080-1657000
BAF Arnot-Gobas method (upper trophic) exc. biotrans. =577200-10600000

Further, based on estimates using KOAWIN v1.10 the logKoa value for monomethyl ether TBBPA is 11.2-14.4 depending on logKow. The estimated logKoa-value fulfils another screening trigger for potentially bioaccumulative substances in the terrestrial environment, unless significant biotransformation (metabolism) occurs, as the estimated logKow values > 2 and the estimated Log Koa values > 6 (Kelly *et al.*, 2007).

Thus the transformation product of TBBPA, monomethyl ether TBBPA fulfils the screening criterion for bioaccumulative and/or very bioaccumulative substances, B/vB (ECHA 2014).

[8] Based on log Kow value of 5.47-8.23

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Toxicity

No measured toxicity data in aquatic or mammalian species have been identified for monomethyl ether TBBPA.

PBT screening

As QSAR data can only be used as a screening tool, further information on the environmental degradation rates of the transformation product is needed in order to first clarify whether the substance meets persistency part of the vPvB/PBT criteria in REACH Annex XIII. In accordance with the general PBT testing strategy focus will namely first be placed on obtaining environmentally relevant degradation data on the two methylated ether TBBPA transformation products to take the 3 R principles into account in the best way possible (i.e. minimisation as much as possible usage of laboratory animal testing). In respect to selection of relevant types of simulation degradation test types (surface water, sediment and/ or soil) account will be taken of the estimated environmental fate properties, as well as the current knowledge about the environmental occurrence. Furthermore, account will also be taken of interpretation problems related to likely outcomes in various types of simulation degradation tests (in particular in respect to the difficulties related to interpretation of NER / BR - formation).

Considerations on the test method and testing strategy

The P and vP properties of the TBBPA degradation metabolite, monomethyl ether TBBPA was screened according to Annex XIII 3.1.1. The results indicate that monomethyl ether TBBPA may be persistent or very persistent under relevant environmental conditions. Further information is needed in order to conclude that the P/vP criterion according to Annex XIII, 1.1.1 / 1.2.1 of the REACH Regulation is met because monomethyl ether TBBPA also meet the B/vB screening criterion according to PBT GD Chapter R.11 hence is a potential PBT or vPvB.

It is acknowledged that the fulfilment of the B screening criteria is based on QSAR estimated logKow values and that the predictions vary. It is however noted that all the predicted logKow values significantly exceeds the screening B-trigger value of logKow=4.5 and that it is also in comparison with the measured logKow value of 5.9 for TBBPA unlikely that the measured value for the monomethyl ether TBBPA would be lower than 4.5. Hence it has been decided not to request a measured logKow at this stage.

Monomethyl ether TBBPA has been shown to be formed from TBBPA in both surface water (Peng *et al.*, 2014), water covered and dry soil (Sun *et al.*, 2014; Li *et al.*, 2015) and sediment under aerobic conditions (George and Häggblom, 2008).

Surface water is an important receiving compartment of TBBPA because of the direct emission of sewage treatment plant effluents to surface water (EU RAR, 2008). Removal of TBBPA in sewage treatment plants (STP) using EPI v. 4.10 predicts a high adsorption (91%) to sludge and low biodegradation (<1%). As data from available studies showed that TBBPA is transformed after 10-35 days to methylated transformation products (Sun *et al.*, 2014) the adsorption to sludge of TBBPA is relevant for the occurrence and environmental distribution of the methylated transformation products. For monomethyl ether TBBPA, 87-93% is predicted to sorp to sludge. Due to sludge disposal this identifies soil as a relevant compartment of concern. However, based on the EPI v.4.10 STP model estimate up to 9% of the mass fraction of TBBPA is being discharged from the

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STP to surface water why surface water also will be the relevant compartment of concern where TBBPA may be methylated to mono- and bismethyl TBBPA either directly in the water phase (including in adsorbed to SPM) or in sediments after sedimentation of POM in sedimentation zones.

Monitoring data of the methylated metabolites of TBBPA in water has been provided by you ([redacted]). The presence of monomethyl ether TBBPA in Suspended Particulate Matter (SPM) was analysed in composite samples from the rivers Western Scheldt (The Netherlands, four samples), Tees and Mersey (Great Britain, four samples and one sample), Rhone (France, four samples) and Götaälv (Sweden, one sample). Sediment was samples from Lake Nalau (Germany, two samples). Monomethyl ether TBBPA was found in all samples from Tees (2.85-3.36 µg/kg dw), Mersey (4.48 µg/kg dw), Rhone (2.59-3.85 µg/kg dw), and Götaälv (2.32 µg/kg dw). Monomethyl ether TBBPA was detected, but not quantified in one sample out of four in Western Scheld. (limit of detection (LOD): 0.2 µg/kg dw, limit of quantification (LOQ): 0.8 µg/kg dw). Monomethyl ether TBBPA was also found in two sediment samples (5.16-5.51 µg/kg dw) from Lake Belau (Germany). No monitoring data of monomethyl ether TBBPA has been found in the open literature.

In conclusion surface water, sediment and soil compartments are all of concern as regards to the environmental persistency of monomethyl ether TBBPA.

In water-sediment studies with the registered substance, TBBPA dissipated from the water phase and adsorbed to the sediment phase with a high amount of bound residues (approximately 50 % ([redacted])) making interpretation of the results difficult. Monomethyl ether TBBPA is also expected to bind significantly to soil, sediment and particular matter (calculated logKoc 4.1-5.7) and, due to its higher logKow value, even to a higher degree than TBBPA due to the additional methyl group. To assess persistence it is necessary to differentiate between mere elimination and degradation processes (cf. REACH Guidance R 11.4.1.1). Thus, a test system where non-extractable residue (NER)-formation is high will cause challenges for test data interpretation. Hence from this perspective sediment and soil degradation data which are likely to include high NER/bound residues (BR) concentrations may be a challenge to interpret.

In the OECD TG 309, the test system provides simulation degradation data on primary degradation or mineralization in surface water (including naturally occurring SPM) depending on the actual testing set up. It either uses surface water only, or can optionally be set up with artificial addition of suspended sediment particles imitating surface waters with high naturally occurring particulate organic matter (suspended sediment test) such as emission zones of STPs or estuaries (both types of surface water with high particulate organic matter). It is not certain whether adsorption to particles would increase or decrease the degradation rate.

Monomethyl ether TBBPA is expected to be found in different types of surface water, including surface waters with high and low content of SPM. You have commented that as monomethyl ether TBBPA will be released as a degradation product of TBBPA from suspended particles in the surface water the requested OECD 309 test (kinetic part) should be performed with addition of suspended sediments/solids in a concentration simulating typical conditions in natural surface waters if simulation degradation testing in surface water proves technically feasible. Based on a proposal for amendment from a MSCA ECHA does not agree that the test should be done with artificially added

particulate material but rather by use of a surface water with a naturally occurring SPM concentration.

Requesting another type of surface water test has also been considered, i.e. a test of the transformation in the open sea. A relatively high environmental transport potential of monomethyl ether TBBPA was predicted by the OECD LRET Model ver. 2.2[9] providing an estimate[10] of overall persistence, POV, of 259 days, a critical travel distance, CTD, of 1243-2854 km, and travel estimation, TE, value of 5.5-12.6%. But as the model output estimates are heavily sensitive of especially the input data for persistency in the environmental media which is target for the present requests, this has not been pursued further at this stage.

Therefore, kinetic degradation half-life data from an OECD TG 309 test using surface water samples with approximately 15 mg SPM dw/L giving degradation half-lives for monomethyl ether TBBPA in relevant EU surface waters is needed as described below:

Aerobic mineralisation in surface water – simulation biodegradation test, EU C.25/OECD 309, pelagic test – with additional suspended solids/sediment particles

As the water solubility of the substance is expected to be low (0.11-24 µg/L based on QSAR predictions based on EPIWIN v. 4.10) a reliable measured water solubility value should be made available before an OECD TG 309 (kinetic part only) is decided and carried out. The reason is that it is important that the initial concentration of the substance in the test water does not exceed the water solubility (request described in section 1.3 and in Endpoint 3) and furthermore that the decrease in the water concentration of the substance can be followed over the 60 days (or more) test period. Unless it can be justified convincingly that a surface water simulation transformation test is unfeasible due to technical/analytical reasons such a test is requested.

The following conditions of the surface water simulation testing should be fulfilled:

- Monomethyl ether TBBPA shall be radiolabelled due to its low water solubility and the consequentially low initial concentration of the substance in the test. You shall provide justification for the location of the radiolabel on the molecule.
- The test should performed under dim light or in the dark.
- The test guideline OECD 309 stipulates test duration of 60 days. If less than 50% of the substance has been degraded at this point the test duration should be extended until > 50 % has been degraded or a test duration of 90 days – whichever comes first.
- The REACH Guidance (cf. Table R.16-9) defines the average default environmental temperature for the EU as 12°C and this is the reference temperature for the assessment of persistency in PBT/vPvB assessment. In order to achieve this, you are requested to perform the test at 12°C (285K) with no need for an Arrhenius temperature normalization, which would be needed if the test was performed e.g. at room temperature.
- In order to increase the analytical capabilities to identify/characterise and quantify the major transformation products it is acceptable if higher concentrations of test

[9] http://www.oecd.org/chemicalsafety/risk-assessment/oecdpovandlrtpscreeningtool.htm
[10] Input: Molecular weight: 55.9 g/mole, logKaw: -8.271 (based on EPIWIN v.4.1), logKow: 5.47 (low) or 8.23 as described in Endpoint 3, Half-life in air:32 h (based on AopWin v. 1.92), Half-life in water: 1440 h and half-life in soil 4320 h (based on the assumption that monomethyl ether TBBPA meets the criteria for P).

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substance are used in the degradation pathway study in accordance with OECD TG paragraph 5. This part of the study may be performed at 20°C.

- Sufficient measurements shall be performed to enhance the possibility of establishing a reliable kinetic modelling. The guideline OECD 309 stipulates that a minimum of 5 sampling points are required during the degradation phase. This refers to the test duration of 60 days, or 90 days. A tight pattern of measurements at 1, 6, 12 and 24 hours and at day 7, 14, 28 and 56 and at the end of the test shall be made. If the test is longer than 60 days measurements should be made at regular intervals thereafter but for no longer than a month in agreement with the OECD 309 guideline, which states that more measurements can easily be done although it does not give a fixed time schedule.
- If technical feasible the primary degradation of the substance and the increase of CO_2, using $^{14}CO_2$ traps, should be measured.
- In order to ensure that it is possible to eliminate adsorption from degradation, recovery of radiolabelled ^{14}C should be stated and evaluated as stipulated in the in the guideline.
- Based on your comments, the pathway (metabolism) part of the study shall not be conducted at this stage if the kinetic study clearly indicates that the primary degradation half-life in surface water of monomethyl ether TBBPA is > 60 days (i.e. the substance fulfils definitively the surface water vP criterion of REACH Annex XIII).
- If the ultimate degradation is complete within 40 days the pathway part of the study is also not requested as this indicates that neither monomethyl ether TBBPA nor its degradation metabolites are persistent in surface water.
- It is possible that the monomethyl ether TBBPA in water with suspended particles may form NER. You requested to justify scientifically that the extraction procedure/solvent chosen is appropriate to completely extract the non-irreversible bound fraction of the substance/its metabolites from the SPM matrix. Strong extractions, such as soxhlet-extraction with apolar solvents, should be used in order to conclude that the remaining part should be considered as NER.
- The water samples used for the surface water simulation degradation test should contain ~15 mg SPM dw/L. Water containing between 10 and 20 mg SPM dw/L is considered acceptable.

Monomethyl ether TBBPA will primarily be released to surface water as a degradation product of TBBPA from suspended particles in the surface water and from the sediment. This was also evident in the monitoring results submitted by you ([redacted]). Based on comments from you, the requested OECD 309 tests shall be conducted with SPM to simulate the typical conditions in natural surface waters in EU.

As stated in the OECD TG 309 (paragraph 5) "The test is performed in batch by incubating the test substance with either surface water only ("pelagic test") or surface water amended with suspended solids/sediment of 0.01 to 1 g/L dry weight ("suspended sediment test") to simulate a water body with suspended solids or re-suspended sediment. The suspended solids/sediment concentration in the lower range of this interval is typical for most surface waters."

It is further specified in Guidance on information requirements and Chemical Safety Assessment Chapter R.16: Environmental exposure assessment (ECHA 2016) that the default SPM concentration for EU fresh surface waters is 15 mg SPM dw/L. The amount of SPM should be present in the sampled water phase, without the addition of extra suspended particles to the test system. If coarse filtration as described by the OECD 309

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removes too much of the amount of SPM, it can be considered to use non-filtered water samples or even more coarse filters.

In your comments you highlighted that "*The monomethyl ether TBBPA is not released into the environment as such but is a transformation product formed mainly by sediment organisms. As a result of sediment re-suspension, it can be expected to be bound to suspended solid particles and not likely to be found in a soluble form in the water*". ECHA agrees that monomethyl ether TBBPA is likely to be most extensively formed in the sediment and on SPM in surface water as TBBPA is expected to be adsorbed to organic material. Hence, a much higher concentration of TBBPA will be present in the sediment and SPM than in true dissolved phase in the water. Based on your comments and these further considerations it is considered that it will be relevant to test the persistency of monomethyl ether TBBPA in surface water containing SPM.

The evaluating MSCA must have access to the robust study summaries as well as the full study report including all relevant details of the study, ensuring that a clear conclusion regarding the result of the study can be drawn by the evaluating MSCA. The reason for requesting the full study report is that its accessibility to the evaluating MSCA is most probably needed in order to evaluate all study details relevant for the result because such details are, based on general experience on higher tier test, not always available in robust study summaries only.

Should it be proved technically unfeasible to perform the water degradation test a sediment simulation degradation test will be needed as described below:

Aerobic and anaerobic transformation in aquatic sediment systems, EU C.24/ OECD 308.

Monomethyl ether TBBPA is predicted to be highly adsorptive and therefore adsorb to a high degree to also sediments, and suspended particles in surface water that in sedimentation areas are deposited naturally on the bottom. A high degree of non-extractable residues (NER) is expected to be generated when testing the monomethyl ether TBBPA in the OECD 308 which may cause challenges for proper test data interpretation. Therefore monomethyl ether TBBPA shall be radiolabelled. You shall provide justification for the location of the radiolabel on the molecule.

The following conditions of testing shall be fulfilled:

- Monomethyl ether TBBPA shall be added to the sediment and not to the water phase as this test will be performed if the substance is very poorly soluble in water.
- The test substance shall be radiolabelled due to its very low water solubility. You shall provide justification for the location of the radiolabel on the molecule.
- As monomethyl ether TBBPA is not expected to be formed under anaerobic conditions the exclusively anaerobic version of the OECD 308 is irrelevant.
- The test guideline OECD 308 stipulates test duration of 100 days. If less than 50% of the substance has been degraded at this point the test duration should be extended until > 50 % has been degraded. Experience[11] shows that an extension to 180 days is possible without reducing significance of data even though the test guideline states that test duration normally should not exceed 100 days.
- Measurements shall be done to model the degradation kinetics. The guideline

[11] R&D projects 20667460/03 and *22801*, UBA 2012 and 2013

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OECD 308 stipulates that the number of sampling times should be at least six including zero time for a test duration of 100 days. This is insufficient for a difficult to test substance like monomethyl ether TBBPA, which is expected to adsorb rapidly to sediment. The test regime shall be such that it is possible to follow the adsorption process over time. This is a necessary provision for a successful kinetic modelling when performing the data evaluation because it may be necessary to re-calculate the test concentration and to adequately identify the point in time to use as the starting point the calculation of the half-life. For being able to do this three samples shall be taken on the first day, after 1 hour, 6 and 12 hours; another sample shall be taken after 24 hours followed by sampling times at day 7, 14 and day 28. The following sampling times shall be nearly evenly distributed in a 4 weeks interval. Hence, depending of the total duration of the study, a total of at least 11 sampling time points for a test duration of 180 days shall be included in the study.

- The primary degradation and the ultimate degradation by measuring the CO_2 generation, using $^{14}CO_2$ traps, should be measured.
- In order to ensure that it is possible to discriminate adsorption from degradation, recovery of radiolabelled ^{14}C should be stated and evaluated as stipulated in the in the guideline.
- Based on your comments, the pathway (metabolism) part of the sediment simulation degradation study does not need to be conducted at this stage if the kinetic study clearly indicates that the degradation half-life in sediment of monomethyl ether TBBPA is > 180 days (i.e. the substance fulfils definitively the sediment vP criterion of REACH Annex XIII).
- If the ultimate degradation is complete within 120 days the pathway part of the study is also not requested.
- The REACH Guidance (cf. Table R.16-9) defines the average environmental temperature for the EU as 12°C and this is the reference temperature for the assessment of persistency in PBT/vPvB assessment. The study shall be performed at 12°C (285K) with no need for an Arrhenius normalisation.
- In order to increase the analytical capabilities to identify/characterize and quantify the major transformation products it is acceptable if a higher concentration of test substance is used in the degradation pathway study in accordance with OECD TG paragraph 34. This part of the study may be performed at 20°C.
- It is possible that the monomethyl ether TBBPA in sediment or soil may form NER. You are requested to justify scientifically that the extraction procedure/solvent chosen is appropriate to completely extract the non-irreversible bound fraction of the substance/its metabolites from the soil/sediment matrix when testing the degradation in these compartments. Strong extractions, such as soxhlet-extraction with apolar solvents, should be used in order to conclude that the remaining part should be considered as NER.

All of these aspects are needed for the interpretation of the processes observed.

The evaluating MSCA must have access to the robust study summaries as well as the full study report including all relevant details of the study, ensuring that a clear conclusion regarding the result of the study can be drawn by the evaluating MSCA. The reason for requesting the full study report is that its accessibility to the evaluating MSCA is most probably needed in order to evaluate all study details relevant for the result because such details are, based on general experience on higher tier test, not always available in robust study summaries only.

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Alternative approaches and Proportionality of the request

Based on the evaluation of all available information on the registered substance further information is required in order to complete the evaluation of whether the substance is PBT or vPvB.

In the studies included in the registration dossier monomethyl ether TBBPA was not identified ([redacted]), however, several unidentified transformation products were detected. In pathway studies from the open Literature monomethyl ether TBBPA has, however, been identified as a transformation product of TBBPA under aerobic conditions. It is your duty to perform PBT assessments of all transformation products/metabolites formed in >0.1%, or to justify why this is not relevant. It should be noted that these newer studies from the open literature have been included in the updated registration dossier(s), however a full assessment based on these studies is still not provided.

Due to proportionality, a sequential approach is requested in accordance with the PBT guidance document (ECHA 2014) where first degradation and the fulfilment of P and/or vP is tested. More data is required to conclude whether the P/vP criterion for surface water, sediment or soil according to Annex XIII, 1.1.1 / 1.2.1 of the REACH Regulation is met. If P or vP can be concluded, an appropriate test of bioaccumulation of the mono methyl ether TBBPA may be requested in a later decision.

If it should prove technically unfeasible to perform the OECD TG 309, the OECD TG 308 sediment simulation test on aerobic and anaerobic transformation in aquatic sediment systems is requested instead. The test has to be performed in accordance with the relevant test guideline.

For both monomethyl and bismethyl ether TBBPA soil, water covered soil, and sediments are compartments of concern. However, taking into account that monomethyl and bismethyl ether TBBPA may have somewhat similar degradation potential (the latter probably due to its higher logKow having a longer degradation half-life) it is deemed proportionate in relation to the testing costs for you to not request the soil simulation degradation test for monomethyl ether TBBPA even though this is also regarded to be relevant. Degradation in the terrestrial environment will instead be addressed by the request for further degradation information on bismethyl ether TBBPA. See also similar and further explanations provided below in subsection 6 as regards the environmental fate of TBBPAs transformation product bismethyl ether TBBPA.

Consideration of your comments

You argued that the relevance of a study in water without the addition of suspended solid particles is questionable, as monomethyl ether TBBPA is not released into the environment as such, but is a transformation product formed mainly by sediment organisms. Thus, it will be expected to be bound to suspended solid particles and not likely to be found in a soluble form in the water. Based on this, you argued that the protocol simulating the situation for the formation of monomethyl ether TBBPA best, is the test with suspended solids. Furthermore, you argued that in this particular situation, the OECD 308 test is more relevant than the OECD 309.

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TBBPA has been shown to be formed in laboratory tests in surface waters, soils, water covered soils, and sediments. Furthermore the environmental fate modelling and available environmental monitoring studies on TBBPA indicate as summarised in this decision that the substance will reach the surface water, soil and sediment compartment. However, as monomethyl ether TBBPA will be released as a degradation product of TBBPA from suspended particles in the surface water and from the sediment, the ECHA agrees with you that the two requested OECD 309 tests (kinetic part) could be done with addition of suspended sediments/solids but in a way which is simulating typical conditions of natural surface water bodies in the EU.

You stated that the degradation pathway of TBBPA is rather complex and most studies looking into it identify the debromination of TBBPA, followed by mineralization of the phenolic ring, as the major degradation pathway. You claimed that methylation only occurs to a minor extent and is dependent on the experimental conditions why an exact quantification as percentage of parent compound is very difficult and would have to take into consideration the kinetics of the different pathways and distribution of the reaction products. The elucidation of the most relevant pathway in the environment would contribute to the discussion and understanding of the fate of TBBPA.

As described in this decision, methylation is considered a relevant pathway. As no simulation degradation studies according to relevant OECD/EU standard test methods have identified the transformation products it is not possible to give an exact quantification of the formation of methylated TBBPA as percentage of parent compound but an estimation based on data from the studies in the open literature has been performed by the evaluating MSCA and is reflected in the decision text. Based on this it seems that much higher percentages than that triggering PBT-assessment (0.1 %) of the methylated degradation metabolites have been shown to be formed under different environmentally relevant conditions.

You questioned the results of the laboratory experiments with sediments and isolated bacteria (by George and Häggblom) as they were conducted with high concentrations of TBBPA at temperatures range of 20°C to 34°C and under conditions that eliminate other pathways that occur under environmental conditions and at relevant concentrations.

ECHA acknowledges that the part of the study where isolated bacteria are used most probably yield accelerated microbial transformation due to particular laboratory conditions. However, this part of the study is not used for deriving degradation kinetics but rather used to identify the degradation pathway and in particular that microbial O-methylation of TBBPA is plausible. Together with other available evidence this study brings together strong evidence that monomethyl- and bismethyl ether TBBPA are products of microbial O-methylation of TBBPA and this transformation is likely to occur under environmental conditions.

You commented that although in the figures in Li *et al.* (2015) there is no differentiation between the two methylated forms, it is indicated in the text that the bismethyl ether TBBPA was detected only on day 143 of the incubation. It should be noted that this study is performed by the same lab/authors.

ECHA does not agree with this interpretation as the authors only suggest that bismethyl ether was not formed within the first 20 days.

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You highlighted that in the [redacted] report, the levels monomethyl ether TBBPA was either below the LOD (0.2 µg/kg ww) or below the LOQ (0.8 µg/kg ww), in 27 out of 37 samples in [redacted] rivers.

ECHA notes that monomethyl ether TBBPA was detected in bream muscle tissue in 0/7 ([redacted]), 2/4 ([redacted]), 3/4 ([redacted]), 4/7 ([redacted]), 7/7 ([redacted]) samples. Monomethyl ether TBBPA was also detected in bream muscle tissue in 7/7 samples ([redacted]). Furthermore, monomethyl ether TBBPA was detected in sole muscle tissue in 7/7 samples ([redacted]). ECHA further notes that monomethyl ether TBBPA was detected in the muscle tissue even though it would be expected to be accumulated in lipid rather than muscle (you have not provided an explanation why muscle and not fatty tissues was used in these monitoring studies conducted/sponsored by you). In conclusion based on limited environmental biomonitoring information monomethyl ether TBPA has been detected in different fish species over several years in several EU surface water bodies in various EU countries. Hence no changes were made in the decision text based on your comments.

You questioned the relevance of the pathway part of the degradation study. You argued that persistence assessment of the monomethyl ether TBBPA is needed for the PBT assessment of the transformation products per REACH annex XIII. However, you perceived investigating further the transformation products of a transformation product as disproportionate and not relevant.

ECHA does not agree that it is disproportionate to request further identification and/or characterization (related to chemical identity and PB properties) of degradation products as the Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment does not refer to primary metabolites only.

You stated in your comments that if the request to investigate the transformation products of the monomethyl ether TBBPA is taken forward, then the study on identification of degradation products should be done only if significant degradation is observed during the study addressing the degradation rate.

ECHA has partly accepted these comments and now accepts the pathway (metabolism) part of the requested sediment simulation degradation study to not be conducted at this stage if the kinetic part of the study indicates that the degradation half-life in surface water of monomethyl ether TBBPA is > 60 days and equivalently as regards the sediment simulation test if the degradation half-life in sediment of monomethyl ether TBBPA is > 180 days (i.e. the substance fulfils definitively the vP criterion of REACH Annex XIII for the respective environmental compartment). The original request to also accept that the pathway part of the study is omitted is kept in the decision i.e. if the ultimate degradation (mineralisation) is less than 40 days (surface water) and 120 days (sediment), because this indicates that neither monomethyl ether TBBPA nor its degradation metabolites are persistent in these respective environmental compartments. Hence the degradation pathway part of the simulation degradation tests on surface water or alternatively on sediments (as described above) is only now requested if needed for the further vPvB/PBT assessment of degradation metabolites of monomethyl ether TBBPA.

You proposed to include the anaerobic part of the OECD 308 study since it is expected that the anaerobic sediment will be a sink for the monomethyl ether TBBPA. You argued that anaerobic part of the OECD 308 will complement the aerobic part to cover the distribution of the monomethyl ether in the sediment compartment.

ECHA has no objection if you also perform the strict anaerobic version of the OECD 308 but do on the other hand not see any reason to do so, as this will not affect the P-evaluation in the context of PBT properties. Anaerobic conditions do normally occur in the deeper layers of a normal sediment degradation study whereas aerobic conditions prevail in the sediment surface layer and in the overlying water. This aerobic/anaerobic sediment transformation study (OECD TG 308) is the one which generally is assumed to represent the degradation in sediments of water bodies and hence which may be required as appropriate under PBT assessment and testing. Hence, the evaluating MSCA did not propose to include the strict anaerobic part of the study in the request in order to save time and money for you. In the requested study the headspace will be filled with air, thus water and the upper layer of the sediment will be aerobic. However, a redox gradient will most probably be established in the test sediment and anaerobic conditions are expected in the test system in the lower part of the sediment.

As further non-animal testing studies do not need approval from authorities, you can do additional studies beyond what is requested in this regard in the current decision, including to conduct also an exclusive anaerobic degradation study, which the evaluating MSCA does not foresee to deliver essential results related to the further PBT/vPvB assessment.

The decision was not changed based on these comments from you.

You stated that approaches to determine the fraction of bound residues that are unlikely to be re-mobilized and thus can be regarded as degraded have been developed and some research is still in progress. These approaches can be applied in the case of NER formation and an interpretation should be possible. (ECETOC 2013 a,b,c). Hence you felt that NER should not be used as an argument to not use the most relevant test system.

ECHA highlights that it is not a question of identifying "the most relevant test system", but rather to identify relevant test system(s). In this case, as indicated above, relevant test systems would include sediment, surface water and soil. By requesting a simulation test in surface water if technically feasible, test result interpretation challenges will be avoided regarding NER, which is likely to be formed. Hence the testing request as modified above is maintained.

You argued that for comparison to the P criteria of REACH Annex XIII a temperature of 20°C should be used as this has been the basis for the criteria. As 12°C according to the REACH endpoint specific Guidance Document for PBT assessment (R11) is the relevant default temperature for simulation degradation testing in Europe the temperature requirement has been maintained.

You agreed to the PfAs which stated that only one SPM concentration (standard EU freshwater concentration) should be used as this make interpretation of the results easier. The decision was amended based on this.

Conclusion

Therefore, based on the substance evaluation and pursuant to Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following studies **using the transformation product monomethyl ether TBBPA (No CAS available):**

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Simulation testing on ultimate degradation in surface water. Test method: Aerobic mineralisation in surface water – simulation biodegradation test, EU C.25/OECD 309 at 12°C (285K), with suspended solids/sediment (~15 mg SPM dw /L)

OR if it can be convincingly justified that these tests are technically/analytically not feasible:

Sediment simulation testing. Test method: Aerobic and anaerobic transformation in aquatic sediment systems, EU C.24./OECD 308 at 12 °C using the transformation product of the registered substance: monomethyl ether TBBPA (No CAS available)

Test conditions are specified above.

ENDPOINT 6: PBT properties: Environmental fate of the transformation product TBBPA bismethyl ether: Simulation degradation testing

The evaluating MSCA has identified a concern for the environmental fate of the methylated transformation product of TBBPA, TBBPA bismethyl ether (CAS 37853-61-5).

In the EU RAR (2008) of TBBPA it was stated that "*Another possible metabolite/ degradation product – tetrabromobisphenol-A bis(methyl ether) – possibly meets the screening criteria for a PBT substance using mainly estimated data. The presence of this substance has been investigated in some recent studies of anaerobic transformation in freshwater aquatic sediment and sewage sludge, and anaerobic and aerobic soil transformation. Although inconclusive, the results suggest that it is a very minor degradation product. Given that a need for risk reduction measures has already been identified for some uses (which should reduce the environmental burden of the parent compound), no further specific work is recommended to address this issue at the present time (conclusion (i) on-hold).*" New studies from the open literature have since proved that significant amounts of TBBPA could be converted to this transformation product. These are described in Endpoint 5.

In the EU RAR it was stated that the transformation product possibly meet the screening criteria for PBT assessment. Available studies from the open literature, published after the finalisation of the EU RAR, show that O-methylating of TBBPA is an important degradation pathway under aerobic conditions and likely to occur in both water, sediment and soil - forming 10%-60% total methylated transformation products. From the current literature it is plausible that up to 10% of naturally occurring bacteria in the environment are able to transform TBBPA to its O-methylated transformation products under aerobic conditions.

The available studies indicate that the methylated transformation products of TBBPA, including bismethyl ether TBBPA, are persistent and are formed at significant levels far exceeding the 0.1% trigger relevant for PBT assessment of transformation products.

Why new information is needed

The evaluating MSCA has identified a concern for the possible PBT properties of the transformation products of the registered substance; bismethyl ether TBBPA.

No test data on the potential biodegradation of bismethyl ether TBBPA has been found.

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Persistency

However, data from soil and sediment studies indicate persistency in the environment (George and Häggblom, 2008; Sun *et al.*, 2014; Li *et al.*, 2015). As test results are not available, QSAR predictions using BIOWIN 4.10 (US-EPA QSAR package EPIWIN) and the Danish QSAR database[12] are presented below.

Biowin1 (linear model) Probability of Rapid Biodegradation: 0.11
Biowin2 (non-linear model) Probability of Rapid Biodegradation: 0.00
Biowin3 Expert Survey Ultimate Biodegradation: 1.06
Biowin4 Expert Survey Primary Biodeg: 2.41
Biowin5 (MITI linear model) Prob. Biodeg: 0.17
Biowin6 (MITI non-linear model) Biodegradation Probability: 0.01
Danish QSAR database: NRB

Note that BIOWIN 1 & 2 score of < 0.5: predicted not rapidly biodegradable
BIOWIN 5 & 6 < 0.5: predicted Not readily Biodegradable (NRB, according to MITI 1 training set), BIOWIN 3 and 4 gives the ultimate and primary timeframe for degradation (1 years, 2 month, 3 weeks, 4 days, 5 hours). The Danish MCase biodeg. Prediction directly indicated whether RB or NRB (based on mainly MITI data training set).

Biowin sub-models 1 and 2 depict that the substances will not degrade rapidly, supported by the Biowin 5 and 6 predictions and the DK QSAR DB. The persistency screening algorithm: BIOWIN2 and/or BIOWIN 6 < 0.5; BIOWIN 3 < 2.2 (2.7), is furthermore fulfilled (ECHA 2014). The molecular weight of bismethyl ether TBBPA (571.93) is within the molecular weight domain of all the models, for which the lowest MW values vary between 30.02 – 53.06 and the maximum MW values vary between 697.65 – 959.2. Thus, Mono-methylated TBBPA meets the screening criteria for being P/vP.

Bioaccumulation potential

No test data or field investigations of bioaccumulation or food-chain transfer of bismethyl ether TBBPA. The QSAR predictions (using KOWWIN v1.68 EPIWEB 4.1 or Vega 1.1.1,) yield a logKow value of 5.69-8.84. Thus, the estimated logKow values are above the screening trigger value of logKow > 4.5, which is the triggering value for bioaccumulation potential (ECHA, 2014). This is supported by the observed BCF and BAF values[13] predicted by BCFWINNT as presented below:

BCF_{fish} = 1669-2266-L/kg ww
BCF Arnot-Gobas method (upper trophic) inc. biotrans. = 900-15330
BCF Arnot-Gobas method (upper trophic) exc. biotrans. = 979-16910
BAF Arnot-Gobas method (upper trophic) inc. biotrans. = 619300-4223000
BAF Arnot-Gobas method (upper trophic) exc. biotrans. =844800- 4676000

Further, based on estimates using KOAWIN v1.10 the Log Koa values for the bismethyl ether TBBPA is 11.2-14.4 depending on logKow.

The estimated log Koa-value fulfils another screening trigger for potentially

[12] http://qsardb.food.dtu.dk/database/index.html

[13] Based on log Kow value of 5.47-8.23

bioaccumulative substances in the terrestrial environment, unless significant biotransformation (metabolism) occurs, as the estimated logKow values > 2 and the estimated logKoa values > 6 (Kelly *et al.*, 2007).

Thus, the transformation product of TBBPA, bismethyl ether TBBPA has a bioaccumulation potential fulfilling the screening criterion for bioaccumulative and/or very bioaccumulative substances, B/vB (ECHA, 2014).

Toxicity

No measured toxicity data in aquatic or mammalian species have been identified for bismethyl ether TBBPA.

PBT Screening

As QSAR data can only be used as a screening tool, further information on the environmental degradation rates of the transformation product is needed in order to first clarify whether the substance meets persistency part of the vPvB/PBT criteria in REACH Annex XIII. In accordance with the general PBT testing strategy focus will namely first be placed on obtaining environmentally relevant degradation data on the two methylated ether TBBPA transformation products to take the 3 R principles into account in the best way possible (i.e. minimization as much as possible usage of laboratory animal testing). In respect to selection of relevant types of simulation degradation test types (surface water, sediment and/ or soil) account will be taken of the estimated environmental fate properties, as well as the current knowledge about the environmental occurrence. Furthermore account will also be taken of interpretation problems related to likely outcomes in various types of simulation degradation tests (in particular in respect to the difficulties related to interpretation of NER / BR - formation).

<u>Considerations on the test method and testing strategy</u>

The P and vP properties of the TBBPA degradation metabolite, bismethyl ether TBBPA was screened according to Annex XIII 3.1.1. The results indicate that bismethyl ether TBBPA may be persistent or very persistent under relevant environmental conditions. Further information is needed in order to conclude that the P/vP criterion for sediment according to Annex XIII, 1.1.1 / 1.2.1 of the REACH Regulation is met because also bismethyl ether TBBPA also meet the B-screening criteria according to the PBT GD Chapter R.11 and hence is a potential PBT or vPvB.

It is acknowledged that the fulfilment of the B screening criteria is based on QSAR estimated logKow values and that the predictions vary. It is however noted that all the predicted logKow values significantly exceed the screening B-trigger value of logKow=4.5 and that it is also in comparison with the measured logKow value of 5.9 for TBBPA unlikely that the measured value for bismethyl ether TBBPA would be lower than 4.5. Hence it has been decided not to request a measured logKow at this stage.

The formation of bismethyl ether TBBPA has been shown to occur in sediment, soil and water covered soil (George and Häggblom, 2008; Li *et al.*, 2015; Sun *et al.*, 2014).

Surface water is an important receiving compartment of TBBPA because of the direct emission of sewage treatment plant effluents to surface water (EU RAR, 2008). Removal of TBBPA in sewage treatment plants (STP) using EPI v. 4.10 predicts a high adsorption

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(91%) to sludge and low biodegradation (<1%). As data from available studies showed that TBBPA is transformed after 10-35 days to methylated transformation products (Sun *et al.*, 2014) the adsorption to sludge of TBBPA is relevant for the occurrence and environmental distribution of the methylated transformation products. For bismethyl ether TBBPA 90-93 % is predicted to adsorb to sludge. Due to sludge disposal this identifies soil as a relevant compartment of concern. However, based on the EPI v. 4.10 model estimate this, up to 9% of the mass fraction of TBBPA is being discharged from the STP to surface water, the relevant compartment of concern will also be surface water from where TBBPA may be methylated to mono- and bismethyl TBBPA either directly in the water phase (including in adsorbed to SPM) or in sediments after sedimentation of POM in sedimentation zones.

Monitoring data of the methylated metabolites of TBBPA in water has been provided by you ([redacted]). Bismethyl ether TBBPA was detected, but not quantified in SPM in all water samples from Tees (4/4) and Mersey (1/1). Bismethyl ether TBBPA was not detected in Götaälv, Western Scheldt or Rhone River. (LOD: 0.2 µg/kg dw, LOQ: 0.7 µg/kg dw). Bismethyl ether TBBPA was not detected in the upper sediment layer (1 - 2 cm) from two samples from Lake Belau (Germany) was analysed. No bismethyl ether TBBPA was detected (LOD: 0.2 µg/kg dw).

Monitoring data from open literature described that bismethyl ether TBBPA was also found in three out of five effluent samples (0.4-0.6 µg/kg dw) from sewage treatment plants, but was not present in any of the five influent samples (LOD 0.1 µg/kg dw) (de Boer *et al.,* 2002). No bismethyl ether TBBPA was detected in the influent samples for five plants in Baden-Württemberg in Germany (Kuch *et al.*, 2001). However, bismethyl ether TBBPA was detected in five effluent samples (0.33-1.45 ng/l), two upstream samples at (0.42-0.86 ng/l), and one downstream sample (1.06 ng/l) (LOD 0.2 µg/kg dw. for particulates and 0.2 ng/l for the dissolved phase).

Bismethyl ether TBBPA was found in the upper sediment layer (top 1 cm) sediments near a plastics factory in Sweden (Sellström and Jansson, 1995). The concentration of bismethyl ether TBBPA was 24 µg/kg dw in sediment 2 km upstream from the factory and 1,500 µg/kg dw in sediment sampled 5 km downstream from the factory. Recovery of the method was variable between 18 and 72%, with a lower recovery generally being found at lower concentrations. (LOD: 1.9 µg/kg dw).

Bismethyl ether TBBPA were detected in two out of 19 samples (0.2-0.3 µg/kg ww) in sediments from the Scheldt basin, and four out of nine samples (0.1-0.4 µg/kg ww) in river sediments from the Netherlands, but was not detected in 19 samples from the Western Scheldt, nine samples from Dublin Bay/Liffey, four river sediment samples from Ireland and 22 samples from United Kingdom rivers and estuaries (de Boer *et al.*, 2002) (LOD 0.1 µg/kg wet weight or 2.4 µg/kg dry weight).

Bismethyl ether was found in eleven out of twelve of the samples (0.11-1.23 µg/kg ww) in sediments associated with the effluents from waste dumps in Norway SFT (2002). (LOD: 0.9 µg/kg ww).

Overall, the available monitoring data in the aquatic environment support that bismethyl ether TBBPA may occur in surface water SPM, in sediment and in STP effluents. No conclusions can be drawn as regards to occurrence of bismethyl ether TBBPA in soil because no studies targeting soil has been reported, but it seems from the "down the drain" scenario highly likely that the bismethyl ether TBBPA may occur in STP sludge

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amended soils.

In conclusion, surface water, sediment and soil compartments are all of concern as regards to the environmental persistency of bismethyl ether TBBPA. However, bismethyl ether TBBPA has such a low water solubility (see Endpoint 3) that it a priori is assumed that requesting a simulation degradation testing in surface water would not be analytically feasible.

In water-sediment studies with the registered substance, TBBPA dissipated from the water phase and adsorbed to the sediment phase with a high amount of bound residues (approximately 50 % ([redacted]) making interpretation of the results difficult. Bismethyl ether TBBPA is also expected to bind significantly to soil, sediment and particular matter (calculated logKoc 4.1-5.7) and, based on its higher logKow value, even to a higher degree than TBBPA due to the two additional methyl groups. To assess persistence it is necessary to differentiate between mere elimination and degradation processes (cf. REACH Guidance R 11.4.1.1). Thus, this may, in respect to interpretation of sediment and soil simulation degradation tests on bismethyl ether TBBPA, be a challenge. Therefore, attempts should be made to take account of this by using test systems where non-extractable residue (NER)-formation is kept as low as possible (e.g. by including sediments / soils with low organic matter), hence from this perspective sediment and soil degradation data which are likely to include high NER/ BR concentrations may be a challenge to interpret.

Bismethyl ether TBBPA is shown to be formed under aerobic conditions in sediment, soil and water covered soil. Therefore, sediment and dry and water covered soil compartments are of concern for bismethyl ether TBBPA. All scenarios are relevant for the environment. As bismethyl ether TBBPA is expected to adsorb in a higher degree to STP sludge it is expected to be deposited on soil to a higher degree than monomethyl ether TBBPA why it is considered particular relevant to investigate the persistence in soil.

Based on the above, a test to investigate the persistency of bismethyl ether TBBPA in soil as described below is warranted.

Aerobic and anaerobic transformation in soil, EU C.23./OECD 307 at a temperature of 12 °C is required on bismethyl ether TBBPA.

The registered substance TBBPA is highly adsorptive to sludge in STPs which may be deposited onto soil. TBBPA is expected to be methylated to the transformation product bismethyl ether TBBPA under aerobic conditions in soil amended with STP sludge. Bismethyl ether TBBPA is predicted to be highly adsorptive and therefore adsorb to a high degree to soil particles. Hence, a high degree of non-extractable residues (NER) is expected to be generated in the tests, which may cause challenges for proper test data interpretation.

The following conditions of soil simulation degradation testing should be fulfilled:

- The test substance should be added to the soil and not to the water phase as the test substance is expected to be poorly soluble in water.
- The test substance shall be radiolabelled due to its expected high adsorption to organic matter. You shall provide justification for the location of the radiolabel on the molecule.
- As Bismethyl ether TBBPA is not expected to be formed under anaerobic

conditions, only aerobic conditions are relevant. Thus soil shall be aerated with air.

- The test guideline OECD 307 stipulates test duration of 120 days. If less than 50% of the substance has been degraded at this point the test duration should be extended until > 50 % has been degraded. Test duration is preferred to be prolonged to 180 days to facilitate comparison of data with the persistency trigger values. Experience[14] shows that an extension to 180 days is possible without reducing significance of data even though the test guideline states that test duration normally should not exceed 100 days.
- Measurements shall be done for modelling the degradation kinetics. The guideline OECD 307 stipulates that the number of sampling times should be at least six including zero time for a test duration of 100 days. This is insufficient for a difficult- to- test- substance like the bismethyl ether TBBPA, which is expected to adsorb rapidly to soil. The test regime shall be such that it is possible to follow the adsorption process over time. This is a necessary provision for a successful kinetic modelling when performing the data evaluation because it may be necessary to re-calculate the test concentration and to adequately identify the point in time to use as the starting point the calculation of the half-life. For being able to do this three samples shall be taken on the first day, after 1 hour, 6 and 12 hours; another sample shall be taken after 24 hours followed by sampling times at day 7, 14 and day 28. The following sampling times shall be nearly evenly distributed in a 4 weeks interval. Hence, depending of the total duration of the study, a total of at least 11 sampling time points for a test duration of 180 days shall be included in the study.
- The primary degradation and the ultimate degradation by measuring CO_2 generation, using $^{14}CO_2$ traps, should be reported.
- In order to ensure that it is possible to eliminate adsorption from degradation, recovery of radiolabelled ^{14}C should be stated and evaluated as stipulated in the in the guideline.
- The degradation pathway part of the simulation degradation test in soil is only requested if needed for the further vPvB/PBT assessment of degradation metabolites of bismethyl ether TBBPA. If bismethyl ether TBBPA is shown to meet the vP criterion, there would be no significant formation of degradation products in the study and therefore no need to identify and quantify degradation products. If bismethyl ether TBBPA is shown to be not persistent, with complete ultimate degradation within 120 days, then any degradation products can also be considered as not persistent.
- The REACH Guidance (cf. Table R.16-9) defines the average environmental temperature for the EU as 12°C and this is the reference temperature for the assessment of persistency in PBT/vPvB assessment. The study shall be performed at 12°C (285K) with no need for an Arrhenius normalisation.
- It is possible that the bismethyl ether TBBPA in soil may form NER. you are requested to justify scientifically that the extraction procedure /solvent chosen is appropriate to completely extract the non-irreversible bound fraction of the substance / its metabolites from the soil/sediment matrix when testing the degradation in these compartments. Strong extractions, such as soxhlet-extraction with apolar solvents, should be used in order to conclude that the remaining part should be considered as NER.

In order to increase the analytical capabilities to identify/characterize and quantify

[14] R&D projects 20667460/03 and *22801*, UBA 2012 and 2013

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transformation products it is acceptable if a higher concentration of test substance is used in the degradation pathway study in accordance with OECD TG paragraph 2 and 41. This part of the study may be performed at 20°C.

All of these aspects are needed for the interpretation of the processes observed.

The evaluating MSCA must have access to the full study report including all relevant details of the study, ensuring that a clear conclusion regarding the result of the study can be drawn by the evaluating MSCA. The reason for requesting the full study report is that its accessibility to the evaluating MSCA is most probably needed in order to evaluate all study details relevant for the result because such details are, based on experience, not always available in robust study summaries only.

Alternative approaches and Proportionality of the request

Based on the evaluation of all available information on the registered substance, further information is required in order to complete the evaluation of whether the substance is PBT or vPvB.

Studies included in the registration dossier as well as several studies in the open literature prove the transformation of the registered substance TBBPA to bismethyl ether TBBPA. In the studies included in the registration dossier bismethyl ether TBBPA was identified as a transformation product of TBBPA in soil ([redacted]) in addition to detection of several unidentified transformation products in concentrations >0.1%. In the open literature, formation of bismethyl ether TBBPA has been identified in water covered and dry soil, and sediment. It is your duty to perform PBT assessments of all transformation products/metabolites formed in >0.1%, or to justify why this is not relevant.

Due to proportionality, a sequential approach is requested in accordance with the PBT guidance document (ECHA, 2014) where first degradation and the fulfilment of P and/or vP is tested. In order to conclude whether the P/vP criterion according to Annex XIII, 1.1.1 / 1.2.1 of the REACH Regulation is met for bismethyl ether TBBPA more data is required.

For both monomethyl and bismethyl ether soil, water covered soil and sediments are compartments of concern. However, taking into account that monomethyl and bismethyl ether TBBPA may have somewhat similar degradation potential (the latter might however probably due to its higher logKow have a longer degradation half-life and being most adsorptive to organic matter including that in sediment and soil) it is deemed proportionate in relation to the testing costs that testing of monomethyl ether TBBPA focuses on the aquatic environment and that the requested simulation degradation tests for bismethyl ether TBBPA focuses on the terrestrial environment.

Consideration of your comments

You argued that there is no evidence, in particular from the recent monitoring data, that the bismethyl ether TBBPA is formed in the environment to a significant extent and therefore you consider further testing of the bismethyl ether TBBPA not justified as monitoring data from a survey performed/sponsored by you did not detect bismethyl ether TBBPA in SPM in the one sample from River Götälv (SE), the four samples from River Rhone (FR) nor the four samples from Western Scheldt (NL) and that no bismethyl

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ether TBBPA was detected in the two sediment samples from Lake Belau (DE). Bismethyl ether TBBPA was detected in one SPM sample analysed from River Mersey (UK) and in all four SPM samples from River Tees (UK). According to your comments bismethyl ether was only detected and below quantifiable levels in two rivers (LOQ=0.8 µg/kg dw). Therefore, in your view, any realistic potential risk/concern from this transformation product that is hardly found in the environment is questionable and the proposed test is not justified in the framework of a substance evaluation.

ECHA does not agree with this conclusion as bismethyl ether was detected in samples from two of the five locations. At these two locations bismethyl ether TBBPA together with TBBPA and monomethyl TBBPA was identified in all samples each year over several years (UK sites 2008-14) indicating yearlong occurrence of microorganisms which can perform the second methylation reaction from mono- to bismethyl ether TBBPA. At the three locations where bismethyl ether TBBPA was not detected this seems to be related to either a low level of occurrence of TBBPA (parent compound, Western Schelt The Netherlands, i.e. the LoD might not allow identification of bismethyl TBBPA) or might plausibly be related to lack of microorganisms specifically performing the second methylation reaction from monomethyl TBBPA to bismethyl TBBPA (sites in FR and SE). In conclusion limited monitoring information gathered in the period 2008-2014 in several EU countries and recently provided by you also indicate that bismethyl ether TBBPA will be formed by degradation of TBBPA in suspended matter of surface water environments in EU countries. Furthermore, monitoring data described in EU RAR (2008) showed significant levels in both environment and biota.

Bismethyl ether TBBPA is a potential PBT/vPvB and hence, further information requests relating to the concern from this transformation product of TBBPA is therefore justified in the framework of a substance evaluation of TBBPA.

With regard to the relevant compartment, the concern raised in the draft decision is addressing the potential formation of bismethyl ether TBBPA in water covered and dry soil after STP sludge containing TBBPA have been spread on the soil. You argued that based on information that was gathered during the EU risk assessment (2008), TBBPA containing STP sewage sludge is not spread on soil. In addition, based on the data gathered for the Voluntary Emissions Control Action Program (VECAP 2014 report), emissions of TBBPA to soil are in your view controlled by the users of TBBPA in Europe. Therefore you conclude that exposure to soil from TBBPA that would be the precondition for the formation of any methyl ether transformation products, is unlikely to occur. ECHA does not agree with your summary of the EU RAR (2008) in this regard. The following is cited from the EU Risk Assessment:

"The vast majority of tetrabromobisphenol-A is likely to enter soil via adsorption onto and subsequent spreading of sewage sludge, but for uses where atmospheric emissions occur, then these releases can also contribute to the concentrations found in soil over time. In addition, particulate waste containing tetrabromobisphenol-A is predicted to be a direct source of emission to industrial/urban soil." (pp 146)

"[...] some confidential site specific information has been provided for eight of the eleven companies in the EU using tetrabromobisphenol-A as a reactive flame retardant in manufacture of epoxy/polycarbonate resins. The information provided includes details of the amounts of tetrabromobisphenol-A used at the sites and in some cases the number of days of use, information on the emissions to air and the size of the waste water treatment plant. The available information indicates that no sewage sludge from these

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sites is applied to agricultural land, and so this route to soil has not been included in the calculations" (pp 146)

"Tetrabromobisphenol-A has been found to be present in compost derived from kitchen and green waste (extended abstract by Brändli *et al.*, 2006). The study investigated the concentrations of various pollutants in over 80 samples of composts and digestates derived from source-separated green and kitchen wastes from 39 commercial composting and digestion plants in Switzerland. The median concentration of tetrabromobisphenol-A found was 0.51 µg/kg dry weight (range of concentrations was around 0.1-2.3 µg/kg dry weight; all values read from a chart)". (pp 148)

"There are no measured levels for tetrabromobisphenol-A in soil in the EU. There is evidence that tetrabromobisphenol-A is present in sewage sludge in the EU and so is likely to enter into the soil compartment when this sludge is applied to soil, but it is not possible to relate these values directly to the scenarios considered in this assessment." (pp 151)

"One possible explanation for the occurrence of tetrabromobisphenol-A in municipal sewage sludge could be from emissions from articles in use (e.g. volatilisation loss with subsequent condensation on surfaces, particulate loss, etc.). However, other possibilities also exist (for example as indicated in Section 3.1.0.4.2, tetrabromobisphenol-A has been reported to be found in toilet paper). The significance of these sources in relation to the levels found in municipal sewage sludge is not clear". (pp 152)

In summary the EU RAR (2008) includes the available site specific information from some but not all industrial TBBPA user sites. Based on this it was clear that some of those sites did not dispose STP sludge to agricultural land. But as the EU RAR also explains other sources of exposure to soil occur including release of TBBPA to municipal STPs from articles containing TBBPA.

The evaluating MSCA has received the VECAP report from you. However, these data cannot be used to exclude that STP sludge containing TBBPA will be deposited on soil. After receiving your comments the evaluating MSCA contacted you in order for you to provide further data which could substantiate your claim that TBBPA will not enter soil. You replied that you have no further data.

You argued that for comparison to the P criteria of Annex XIII a temperature of 20°C should be used as this has been the basis for the criteria.

As 12°C according to the REACH endpoint specific Guidance Document for PBT assessment (R11) is the relevant default temperature for simulation degradation testing in Europe the final decision has been maintained as regards the request for the studies to be performed at 12 C.

The decision to include a request of degradation simulation studies in soil and water covered soil i.e. that the soil compartment is a relevant compartment for simulation degradation testing was not changed based on your comments. In some PfAs it was proposed to replace the originally requested water covered soil simulation degradation study with a simulation degradation study in sediment. You commented that you agreed with with the PfA submitter that the sediment compartment would be more relevant than the soil compartment. You also agreed with a PfA stating that the water covered simulation degradation test in soil was irrelevant. As the two transformation metabolites, monomethyl ether TBBPA and bismethyl ether TBBPA, are expected to have rather

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similar properties with respect to fate in the environment, ECHA decided to remove the request for a water covered soil simulation degradation study based on the PfAs and your comments on them and not replace it with a simulation degradation study in sediment as monomethyl ether TBBPA will cover the aquatic environment for the two transformation products.

Furthermore, one PfA proposed that the pathway studies for bismethyl ether TBBPA should be conditional in the same way as they were for monomethyl ether TBBPA. You commented that you also agreed that the pathway part of the study should not be investigated if the ultimate degradation half-life in soil/sediments is < 120 days or primary degradation half-life in soils/sediments >180 days. However, you also claimed that you did not understand why the cut-off was chosen to be if the test substance is vP and not just P.

ECHA would like to highlight that this is a special case i.e. this request is on the transformation product of the registered substance and not the registered substance itself. ECHA concluded that should the substance have an ultimate degradation half-life in soil/sediments < 120 days the pathway study would not be relevant as neither the transformation product nor its metabolites would be persistent. To further ease the study for you it was also decided that if the transformation product should have a primary degradation half-life in soils/sediments >180 days the identification/characterisation of the metabolites should not be performed based on all of the three reasons below taken together;

-the pathway part of the study would be technically difficult;

-it would deal with only a secondary degradation metabolite of TBBPA;

-in this particular case it is not highly likely that such a secondary metabolite degradation metabolite would be more persistent than the primary degradation.

Conclusion

Therefore, based on the substance evaluation and pursuant to Article 46(1) of the REACH Regulation, ECHA concludes that you are required to carry out the following studies **using the transformation product bismethyl ether TBBPA (CAS 37853-61-5)**:

Soil simulation testing; test method: Aerobic and anaerobic transformation in soil, EU C.23./OECD 307 at a temperature of 12 °C

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Appendix 2: Procedural history

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to human health; reproduction, endocrine disruptive properties in the environment and human health, exposure, and PBT properties, 2,2´6,6´-tetrabromo-4-4´isopropylidenediphenol (TBBPA) CAS No 79-94-7 (EC No 201-236-9) was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2015. The updated CoRAP was published on the ECHA website on 17 March 2015. The Competent Authority of Denmark (hereafter called the evaluating MSCA) was appointed to carry out the evaluation.

Pursuant to Article 45(4) of the REACH Regulation the evaluating MSCA carried out the evaluation of the above substance based on the information in your registration(s) and other relevant and available information.

In the course of the evaluation, the evaluating MSCA identified additional concerns regarding carcinogenicity based on the 2-year carcinogenicity study performed by the National Toxicology Programme in the Unites States of America (published September 2014).

On 26 February 2016 you updated your dossier based on a compliance check performed by ECHA in August 2014. The update included: new PBT assessment including transformation/degradation products and impurities, an ED review, the NTP 2-year carcinogenicity study, a new GHS self-classification of Carcinogen cat. 2, Revised DNEL calculations, monitoring data for the registered substance and its transformation products, revised Exposure assessment and Exposure Scenario's, revised PNEC calculations and other minor adaptations.

The evaluating MSCA considered that further information was required to clarify the following concerns: endocrine disruptive properties, exposure, and PBT properties. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA on 15 March 2016.

The decision making followed the procedure of Articles 50 and 52 of the REACH Regulation.

ECHA notified you of the draft decision and invited you to provide comments.

Registrant(s)' commenting phase

ECHA received comments from you and forwarded them to the evaluating MSCA without delay.

The evaluating MSCA took into account the comments from you, which were sent within the commenting period, and they are reflected in the Reasons (Appendix 1).

Proposals for amendment by other MSCAs and ECHA and referral to Member State Committee

The evaluating MSCA notified the draft decision to the Competent Authorities of the

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other Member States and ECHA for proposal(s) for amendment.

Subsequently, the evaluating MSCA received proposals for amendment to the draft decision regarding five out of six information requests. They are reflected in the Reasons (Appendix 1).

ECHA referred the draft decision, together with your comments, to the Member State Committee.

ECHA invited you to comment on the proposed amendment(s). Any comments on the proposal(s) for amendment were taken into account by the Member State Committee and are reflected in the Reasons (Appendix 1).

The Member State Committee reached a unanimous agreement on the draft decision during its meeting and ECHA took the decision according to Article 52(2) and 51(6) of the REACH Regulation.

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Appendix 3: Further information, observations and technical guidance

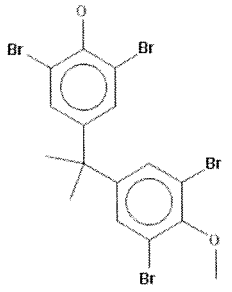
1. This decision does not imply that the information provided by you in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on your dossier(s) at a later stage, nor does it prevent a subsequent decision under the current substance evaluation or a new substance evaluation process once the present substance evaluation has been completed.

2. Failure to comply with the request(s) in this decision, or to fulfil otherwise the information requirement(s) with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

3. In relation to the required experimental study/ies, the sample of the substance to be used shall have a composition that is within the specifications of the substance composition that are given by all Registrant(s).

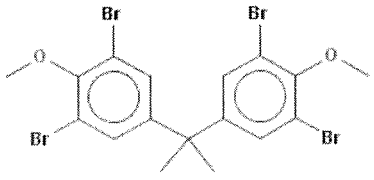
   For the transformation products the following indicators are presented in order to ensure substance identity:

   Monomethyl ether TBBPA:
   SMILES : c1(Br)c(OC)c(Br)cc(C(C)(C)c2cc(Br)c(O)c(Br)c2)c1
   Structure:



   Bismethyl ether TBBPA (CAS)
   SMILES : c1(Br)c(OC)c(Br)cc(C(C)(C)c2cc(Br)c(OC)c(Br)c2)c1

   Structure:



   It is the responsibility of all the Registrant(s) to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating

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MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation.

4. In relation to the experimental stud(y/ies) the legal text foresees the sharing of information and costs between Registrant(s) (Article 53 of the REACH Regulation). You are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who is to carry out the study on behalf of the other Registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at:
https://comments.echa.europa.eu/comments_cms/SEDraftDecisionComments.aspx

Further advice can be found at
http://echa.europa.eu/regulations/reach/registration/data-sharing.
If ECHA is not informed of such agreement within 90 days, it will designate one of the Registrants to perform the stud(y/ies) on behalf of all of them.