



Helsinki, 30 May 2017

Substance name: bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide  
EC number: 201-279-3  
CAS number: 80-43-3  
Date of Latest submission(s) considered<sup>1</sup>: 8 June 2016  
Decision/annotation number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)  
Addressees: Registrant(s)<sup>2</sup> of bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide (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:

1. Simulation testing on ultimate degradation of bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide in surface water (test method: Aerobic mineralisation in surface water – simulation biodegradation test, EU C.25/OECD 309, pelagic test – without additional suspended solids/sediment, containing a natural concentration of ~15 mg SPM dw/l) as specified in Appendix I, section 1. The study shall be performed at 12 °C.
2. In case the study requested under point 1 results in the registered substance to meet the criteria for a persistent (P) or very persistent (vP) substances under REACH Annex XIII, the following study is required: Bioaccumulation of bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide in aquatic species (Annex IX, Section 9.3.2.; test method Bioaccumulation in Fish: Aqueous Exposure Bioaccumulation Fish Test, OECD 305).

You shall provide an update of the registration dossier(s) containing either the information requests of 1 and 2 by **9 December 2019** or the information request of 1 only (if substance is not P or vP) by **7 March 2019** from the date of the decision, including robust study summaries and, where relevant, an update of the Chemical Safety Report, including PBT/vPvB assessment. The deadlines take into account the time that you, the Registrant(s), may need to agree on who is to perform any required tests. They have been set to allow for sequential testing.

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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<sup>1</sup> 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.

<sup>2</sup> The terms Registrant(s), dossier(s) or registration(s) are used throughout the decision, irrespective of the number of Registrants addressed by the decision.



## **2. Who performs the testing**

Based on Article 53 of the REACH Regulation, you are requested to inform ECHA who will carry out the study/ies on behalf of all Registrant(s) 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<sup>3</sup> by Leena Ylä-Mononen, Director of Evaluation

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

## Appendix 1: Reasons

Based on the evaluation of all relevant information submitted on bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide and other relevant available information, ECHA concludes that further information is required in order to enable the evaluating Member State Competent Authority (evaluating MSCA) to complete the evaluation of whether the substance constitutes a risk to 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 the environment (suspected PBT/vPvB).

Bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide is a suspected PBT substance. Further information is needed to assess the P/vP (persistent/very persistent) and B/vB (bioaccumulative/very bioaccumulative) properties as discussed under the relevant testing requirements.

The evaluating MSCA considers that the criteria for toxicity are fulfilled based on the results from a recent OECD 414 study on prenatal developmental toxicity in rats. The study demonstrated that there was an increase in several parameters of reproductive toxicity in the fetuses. There were clinical signs and necropsy findings in some dams indicating maternal toxicity, however these cannot be characterised as "marked". According to the Guidance on the application of the CLP criteria Version 4.1 (ECHA, June 2015) section 3.7.2.2.1.2, 1<sup>st</sup> paragraph, "parental toxicity that is less than marked should not influence the classification for reproductive toxicity independent of the specific parental effects observed". Since the effects in the fetuses cannot be ascribed to parental toxicity, the criteria set out in CLP, EC Regulation (EC) No 1272/2008, Annex I, 3.7.2., for classification of the substance as a reproductive toxicant, Repr 2, is considered by the evaluating MSCA to be fulfilled.

The evaluating MSCA has recently submitted a CLH proposal for the substance based on the existing developmental toxicity study.

You propose to expand the tiered testing approach and start with a prenatal developmental toxicity study (OECD 414) in a second species. You have submitted a testing proposal for such a study which is currently being evaluated by ECHA. Irrespective of the outcome of the examination of the submitted testing proposal, the evaluating MSCA believes that the CLH proposal is justified based on the already existing data.

### Tiered approach for the PBT concern

Bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide is a suspected PBT substance and the criteria for toxicity (T) according to REACH Annex XIII is considered fulfilled. The persistency/very persistency (P/vP) in water shall be determined before starting a possible bioaccumulation study. If bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide is determined to not be P or vP, the bioaccumulation study is not necessary.

**1. Simulation testing on ultimate degradation of bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide in surface water (test method: Aerobic mineralisation in surface water – simulation biodegradation test, EU C.25/OECD 309, pelagic test – without additional suspended solids/sediment, containing a natural concentration of ~15 mg SPM dw/l)**

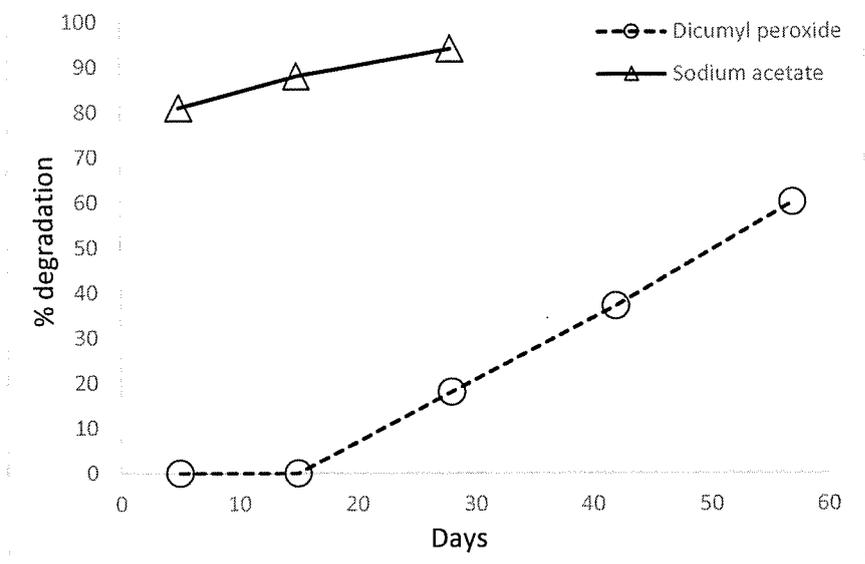
The concern(s) identified and why new information is needed

There is concern that bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide may be persistent in the environment and fulfil the P criteria for PBT according to REACH Annex XIII. Hydrolysis data indicates that the substance is hydrolytically stable at environmentally relevant pH and temperatures.

According to the three screening tests available for biodegradation (OECD 301C, 301D and 301F), it cannot be concluded that bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide is not persistent.

- The OECD TG 301F is a recently performed OECD 301F (2015) manometric test, listed as the key experimental result in the registration dossier. The study uses test concentrations of 20 and 100 mg/l and showed great variability between the replicates. After 28 days, 20% biodegradation (with a standard deviation of 28.8%) was observed for the concentration of 100 mg/l test substance and 44% biodegradation (with a standard deviation of 30.6 %) for the concentration of 20 mg/l test substance. The test does not demonstrate that the substance is readily biodegradable, although some mineralisation was observed.
- The OECD TG 301C study with a test concentration of 100 mg/l showed 0 % biodegradation after 28 days. The test does not demonstrate that the substance is readily biodegradable.
- The OECD TG 301D study was extended to 57 days, and may be considered an enhanced degradation test. The test concentration was 1000 mg/l, and demonstrated 60% degradation by the end of the extended testing period, but only 18% biodegradation after standard testing time of 28 days. As shown in the plot of the degradation data from the 301D test (Figure 1), the degradation is 0% in the first half of the standard test period and later speeds up. In addition, even when the lag phase is considered, the substance still fails the 10 day window as well as 60% degradation in 28 days after degradation starts (Figure 1). The long lag phase and subsequent acceleration in the OECD 301D test suggests that adaptation of the microorganisms may have occurred and the test prolongation should not be considered as adequate for P assessment. It is noted that the lag time varies between the different screening tests and may be a consequence of biological variability.

**Figure 1 - OECD 301D degradation of bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide**



ECHA considers that the available data are not appropriate for dismissing a P/vP concern.

The OECD SIDS document relevant for this substance (COCAM 2012), makes a comparison to another dialkyl peroxide, [1,3(or 1,4)-phenylenebis(1-methylethylidene)]bis[tert-butyl] peroxide (EC 246-678-3, CAS 25155-25-3, undergoing substance evaluation by the Netherlands MSCA), which shows that similar substances are also not readily biodegraded. Bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide is composed of two isopropylbenzoyl groups (cumyl) and [1,3(or 1,4)-phenylenebis(1-methylethylidene)]bis[tert-butyl] peroxide has both a substituted isopropyl benzoyl group and t-butyl functional groups. Both substances contain comparably shielded peroxide groups, with tert-butyl groups shielding for [1,3(or 1,4)-phenylenebis(1-methylethylidene)]bis[tert-butyl] peroxide and cumyl groups shielding for bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide (Antonello et al 1997). They both demonstrate low reactivity at neutral pH and ambient temperatures and are not expected to be highly reactive in the environment.

Based on the results of the screening tests it cannot be concluded that the registered substance is not persistent. Therefore you need to conduct higher tier simulation tests on biodegradation behaviour of the substance to draw a conclusion regarding the P/vP criteria.

#### Considerations on the test method and testing strategy

Information from the simulation test (OECD 309) can directly be compared to the P-criterion for the aquatic aerobic compartment in the PBT assessment, if the test is performed in a way that reflects the environmental conditions of the aquatic aerobic compartment sufficiently well. For persistency there is a need to obtain convincing evidence to be able to conclude about the degradation rate. To this end, a test according to OECD 309 mineralisation in surface water in its pelagic version shall be performed. Non extractable residues (NER) formation in such a test is low, which will minimize any

potential interpretation problems related to the NER formation. NER formation may be expected based on the Log  $K_{oc}$  of the registered substance of 3.98, measured using OECD test guideline 121. Furthermore, the registered substance has been detected in Norway in wastewater treatment plant effluents at low ng/l concentrations, indicating that surface water is a compartment of concern (Miljødirektoratet report M-176. Screening program 2013 - New bisphenols, organic peroxides, fluorinated siloxanes, organic UV filters and selected PBT substances. Published 2014). Studying the degradation in surface water is expected to be technically feasible because the water solubility of the registered substance is 0.43 mg/l.

It is the aim of the request to test the substance in a test system with a small surface area for adsorption. The test system shall be set up to ensure that NER-formation is kept to a minimum. In the OECD TG 309 Guideline two test options, the "pelagic test" and the "suspended sediment test", are described. ECHA considers that the "pelagic test" option should be followed as that is the recommended option for P assessment. The amount of suspended solids in the pelagic test should be representative of the level of suspended solids in EU surface water. The concentration of suspended solids in the surface water sample used should therefore be approximately 15 mg dw/l. Natural surface water containing between 10 and 20 mg SPM dw/l is considered acceptable. Furthermore, when reporting the non-extractable residues (NER) in your test results you should explain and scientifically justify the extraction procedure and solvent used for obtaining a quantitative measure of NER.

It is possible that bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide in water with suspended particular matter 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 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 following conditions shall be fulfilled:

- The initial concentration of the substance in the test water must not exceed the water solubility of the substance in the test system. The registered substance must be radiolabelled due to its low water solubility for an appropriate verification of the degradation kinetics and pathways. You shall provide justification for the location of the radiolabel on the molecule.
- Metabolites shall be identified and sufficiently quantified and characterized as regards their PBT properties (at a concentration of  $\geq 0.1\%$  w/w unless it can be demonstrated that this is technically not possible).
- If relevant, degradants resulting from the reaction with metal ions should also be considered in the simulation study as this potentially appears to be an important degradation pathway in the environment.
- The test guideline OECD 309 stipulates a test duration of 60 days but also states that it may be extended to a maximum of 90 days. It further describes that the test duration may be prolonged to several months if the provisions of Annex 3 of the guideline are followed. Annex 3 describes the semi-continuous procedure which shall prevent deterioration of the system by keeping inoculum viable. However, this procedure includes replacement of water with freshly sampled water and may result in loss of a part of the substance. Hence, account of this should be made either in the procedure of the testing and/ or when evaluating the results of the study. In any case test water renewal shall be started at the

latest possible time (e.g. after 60 days) and the number of subsequent repetitions of water removal shall be restricted to a minimum. It is necessary to closely check the test concentration just before and after each test water renewal if this is employed. All procedures which could complicate the interpretation of results or the extrapolation to the behaviour of the substance in environment, should be avoided as far as possible.

- 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 if a semi-continuous procedure is used. 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.
- The REACH Guidance (cf. Table R.16-9, ECHA, 2014) 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<sup>4</sup>.

#### Alternative approaches and Proportionality of the request

The current concern focuses on the degradation potential in surface water, rather than in sediment or soil. It is necessary to obtain information that will allow the evaluating MSCA to clarify whether bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide is persistent or very persistent according to REACH Annex XIII. More explicitly, there is no equally suitable alternative way available of obtaining this information. The substance has already been demonstrated to not be readily biodegradable in two ready biodegradation tests, and the results from an enhanced biodegradation test was not suitable to dismiss persistency. Further, the substance has been detected in wastewater treatment plant effluent in Norway, indicating that it is released to the environment. Simulation testing is therefore necessary to unequivocally determine its persistence under relevant environmental conditions. Where the data, once obtained, confirms that the registered substance meets the PBT or vPvB criteria, it will allow authorities to consider further regulatory risk management in form of identification as a Substance of Very High Concern in accordance with REACH Article 57.

#### Consideration of Registrants' comments

As mentioned above you propose to expand the tiered testing approach and start with a prenatal developmental toxicity study (OECD 414) in a second species. You have submitted a testing proposal for such a study which is currently being evaluated by ECHA.

For the evaluation of the PBT concern, investigation of P and B properties should be continued on the basis of the existing data which demonstrates concern for these

<sup>4</sup> R.11.4.1.1 *Persistence assessment (P and vP)* in Chapter R.11: PBT/vPvB assessment Version 2.0 (ECHA, 2014): "Please note that since its 32nd meeting the Member State Committee has started to require new simulation degradation studies to be carried out around neutral pH values and at 12 °C, which is understood as the mean temperature of European surface waters. Accordingly, temperature correction of degradation half-lives from already available study results to 12 °C is recommended. In the absence of equations/models reflecting temperature dependence of biodegradation, the Arrhenius equation as provided under the section on "Temperature dependence of hydrolysis" of this Guidance (or a similar appropriate equation designed to normalise physico-chemical degradation rates) can be used as a possible means of normalisation."

properties (see also section *R.11.4.1 Standard approach* in Chapter R.11: PBT/vPvB assessment Version 2.0 (ECHA, 2014)).

For the persistency testing, you suggest a tiered approach with first performing enhanced screening tests and/or inherent biodegradation tests. ECHA does not agree with this. Since three screening tests (two ready biodegradability and one enhanced biodegradability) already have been performed without being able to show that the substance is not P, a simulation test should be performed to allow a definitive conclusion to be made regarding the persistence of the substance. While inherent tests have not been conducted for this substance, ECHA is of the opinion that the potential of screening level test protocols has been sufficiently explored, providing no indication that further testing at a screening level could remove the P concern. Further screening level testing is not justified, and there is a need for a definitive conclusion due to the PBT concern.

You do not agree that the simulation test should be performed at 12°C. Paragraph 24 of the OECD 309 test guideline states that: "*Incubation should take place in the dark (preferred) or in diffuse light at a controlled ( $\pm 2^\circ\text{C}$ ) temperature, which may be the field temperature or a standard temperature of 20-25°C. Field temperature may be either the actual temperature of the sample at the sampling time or an average field temperature at the sampling site.*" A temperature of 12°C is acknowledged to be the mean temperature of European surface waters and can be considered as the average field temperature in this case. It is specified in the REACH Guidance Chapter R.11: PBT/vPvB assessment Version 2.0 (ECHA, 2014) that 12°C is understood to be the mean temperature of European surface waters<sup>5</sup>.

Concerning your claim that the initial step of biodegradation of organic peroxides is their enzymatic cleavage by naturally occurring peroxidases and the need for a literature review to verify this, the evaluating MSCA contracted the University of Oslo to perform a search of the published literature to investigate this further. Their conclusion, following the analysis of the existing scientific literature and websites generally available via the internet, was that no report has been found that describes an enzymatic activity able to metabolize bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide or other aryl/alkyl peroxides, neither in humans, nor in other living organisms.

#### Consideration of Proposal(s) for Amendment (PfA) and Registrants' comments to PfAs

One Member State suggested in their Proposals for amendment (PfA) to perform an enhanced ready degradation study as a first tier and the simulation study being conditional to the outcome of this enhanced test. The Member State also asked for clarification on the reasoning for not considering the OECD 301D test as sufficient to conclude that bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide is not P. In response to the PfA, more clarification on the OECD TG 301D has been provided in the text above. You reiterated your preference for performing a regular inherent biodegradability test in a tiered testing strategy. As further reflected above, ECHA does not agree to this proposal and retains

<sup>5</sup>R.11.4.1.1 *Persistence assessment (P and vP)* in Chapter R.11: PBT/vPvB assessment Version 2.0 (ECHA, 2014): "Please note that since its 32nd meeting the Member State Committee has started to require new simulation degradation studies to be carried out around neutral pH values and at 12°C, which is understood as the mean temperature of European surface waters. Accordingly, temperature correction of degradation half-lives from already available study results to 12°C is recommended. In the absence of equations/models reflecting temperature dependence of biodegradation, the Arrhenius equation as provided under the section on "Temperature dependence of hydrolysis" of this Guidance (or a similar appropriate equation designed to normalise physico-chemical degradation rates) can be used as a possible means of normalisation."



the testing requirements. ECHA considers that any new screening test would not be sufficient to conclude on the P concern.

### 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 registered substance subject to this decision:

Simulation testing on ultimate degradation in surface water (test method: Aerobic mineralisation in surface water - simulation biodegradation test, EU C.25/ OECD 309, pelagic test – without additional solids/sediment, containing a natural concentration of ~15 mg SPM dw/l) conducted at 12°C.

You shall revise the PBT/vPvB assessment when the new information on biodegradation is available.

### **2. Bioaccumulation of bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide in aquatic species (Annex IX, Section 9.3.2.; test method Bioaccumulation in Fish: Aqueous Exposure Bioaccumulation Fish Test, OECD 305)**

This requirement is conditional to the outcome of requirement 1. If it is shown that the registered substance does not meet the criteria for persistent (P) or very persistent substances (vP) under REACH Annex XIII, the information for this endpoint is no longer required.

#### The concern(s) identified and why new information is needed

According to the data in the registration dossiers, the octanol water partition coefficient has been determined to be  $\text{Log Kow} = 5.6$ . This indicates that bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide is likely to partition in the lipids of aquatic organisms.

You have provided a study from 1985 conducted by the Ministry of International Trade and Industry (MITI) in Japan, according to OECD Guideline 305C (Bioaccumulation: Test for the Degree of Bioconcentration in Fish). Carp (*Cyprinus carpio*) were exposed to test substance concentrations of 0.01 and 0.001 mg/l at 25°C for 56 d under flow-through conditions. Reported experimental BCF values (fish) in the registration ranged from 137 and up to 1470, with most of the values falling within the range 337-720. The limited details available and the experimental design used for determining the bioconcentration potential makes it difficult to conclude with confidence whether or not bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide will bioconcentrate in the environment.

Water concentrations are consistent throughout the experiment, as reported in the translated version of the test (Table 1), although the measured concentrations were approximately 50% of the nominal concentration. BCF values seem unstable at the higher concentration, but more stable in the lower (Table 2). The increased BCF values at 4 weeks are not likely to have been caused by unstable concentrations of bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide in water. The BCF data after 4 weeks in the 10  $\mu\text{g/l}$  concentration (i.e. 1440 and 1470) may however be analytical errors, especially if an apparent steady state is assumed after 2 weeks.

Only 2 fish were analysed/concentration/time point. There should be 4 fish analysed per concentration for a valid OECD 305. Actual chemical concentrations of bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide in fish are not reported, only calculated BCFs, which makes calculating a kinetic BCF difficult. The depuration data (Table 3) should be viewed with

caution since they are very variable ranging from 95% residuals to 7.5% after 7 days depuration. These may also be analytical errors, and make calculating the kinetic BCF difficult and very uncertain. Without the kinetic BCF, it is difficult to conclude with any certainty and a BCF test according to OECD is therefore warranted. The 2<sup>nd</sup> concentration (1 µg/l) does not seem to depurate below 20-30 % over the 7 days, but this may be due to the concentration levels being close to the LOQ (the report only seems to detail the LOD).

**Table 1: Concentration of the test substance in test water (measured value); W= week**

	1 W	2 W	4 W	6 W	8 W
10 µg/L - nominal	4.08	3.93	5.04	5.59	5.81
1 µg/L - nominal	0.487	0.474	0.535	0.584	0.602

(unit: µg/l)

**Table 2 – Reported BCF values**

	1 W	2 W	4 W	6 W	8 W
10 µg/l - nominal	137	654	1470	501	711
	468	720	1440	328	703
1 µg/l - nominal	364	520	667	404	337
	181	404	609	505	438

**Table 3 Residual % in fish – depuration phase**

	1 <sup>st</sup> day	3 <sup>rd</sup> day	7 <sup>th</sup> day
10 µg/l - nominal	70.7	37.7	7.46
	30.2	25.6	95.2
1 µg/l - nominal	24.5	25.8	34.8
	13.3	35.0	13.3

The evaluating MSCA has had the original study translated from Japanese. However, important information is still missing from this study report. Of concern is the lack of growth correction and lipid normalization. Food was given twice daily in significant amounts, totalling 4%. Fish growth is expected to be extensive at 3% feeding rate<sup>6</sup> and may have had a significant effect on the reported values through growth dilution. When compiling the information from fish bioaccumulation studies on other substances in table 4 (below), it was noticed that in most of the studies where information on feeding is reported, the feed amount appears to be a total of 2% of the fish body weight, provided in halves twice daily. However, in the Japanese study on bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide it appears that 2% was given twice daily, unless this was an error of translation. The description seems to be very clear<sup>7</sup>, and in addition, the mechanical malfunction at the beginning of the test seems to have been caused by excessive faeces in the tank clogging the system. This seems to support a high level of available food. It is difficult to determine the effect that a doubling of the usual amount of feed may have had on growth in the Japanese study on bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide. A high amount of feed may lead to extensive growth, but may also lead to higher lipid content in the fish. Although the extent of growth or final lipid content is not reported, a calculation has been attempted by the evaluating MSCA using the body weight value of 30 g cited for calculating the detection limit in fish. The average weight at the start of

<sup>6</sup> Draft Validation Report of a Ring Test for the OECD 305 Dietary Exposure Bioaccumulation Fish test, 5th December 2011, <http://www.oecd.org/chemicalsafety/testing/49190726.pdf>

<sup>7</sup> There is no other information concerning feeding in the Japanese bioaccumulation study than this sentence (TRANSLATED): "Feeding: The feed for Carps (made by Nippon Formula Feed Mfg. Co., Ltd.) was given twice daily in significant amount about 2% of fish weight".

the experiment was 22.4 g and the average lipid content was 4.6%. Lipid content is normalized to 5%.

Assuming that lipid content did not change during the exposure period and that 30 g is a realistic average weight at the end of the exposure, a simple calculation suggests that the BCFs may need to be adjusted by:

$$\left(\frac{30g}{22,4g}\right) * \left(\frac{5\%}{4,6\%}\right) = 1,46$$

This adjustment would lead to a bioaccumulation factor for the highest measured BCFs above the B criterion of BCF >2000:

$$1470 * 1,46 = 2140$$

The calculation performed by the evaluating MSCA is intended to demonstrate that there is a concern for BCF in a reasonable worst case scenario and is not based on any guideline or guidance. Whether this actually represents the true BCF for the substance is uncertain. Thus, there is therefore a need to perform testing on bioaccumulation, rather than performing further calculations on a poorly documented set of data.

For the other BCF values of approximately 700 or less, applying the same calculation as above would require the fish to nearly triple in size for the BCF values to exceed 2000. The evaluating MSCA has not been able to find similar studies with fish of comparable sizes and feed amount that also report details on fish growth. Table 4 summarizes the most informative studies found when searching through substances registered with 100+ tonnes on the ECHA disseminated pages, filtered for Carp BCF studies using the QSAR Toolbox. A general finding is that indeed a tripling of size was not seen in BCF studies where carp of comparable size are used. The closest value found is reported for triethoxyoctylsilane, where the fish grew by up to 85%, close to doubling in average size for the low treatment group.

Feeding studies on carp, demonstrate remarkable growth potential for the species, up to tripling in size in one study<sup>8</sup>, albeit with a lower starting weight and higher feeding rate (5%). A second study<sup>9</sup> indicates a growth rate of 2.09% at 24°C and 4% feeding rate, although the initial weight of the fish was 8.73 g. A third study<sup>10</sup>, with fish of comparable size, 27.3 g, reported a daily growth rate of 1,62% after a 120 day growth period, but using 3% feeding rate for the control feed. The UN Food and Agriculture Organization cites a growth rate<sup>11</sup> for *C. carpio* of 2-4% per day (no feeding regime or starting weight specified). A daily growth of 2 – 4% would result in 3 to 9 times increase in weight respectively by the end of the 56 day trial. Although none of the conditions are identical, and the feed composition is not known in some cases, the results from the studies cited indicate that a tripling in size could be within the range of possibilities for carp. However, the likelihood of this happening during a OECD 305 bioconcentration study is debatable.

<sup>8</sup> Sultana et al, 2001, [http://aquaticcommons.org/17788/1/BJFR5.2\\_149.pdf](http://aquaticcommons.org/17788/1/BJFR5.2_149.pdf)

<sup>9</sup> Goolish and Adelman, 1984, <http://www.sciencedirect.com/science/article/pii/0044848684900516>

<sup>10</sup> Gaber, 2009, [http://www.academicjournals.org/article/article1380793654\\_Gaber.pdf](http://www.academicjournals.org/article/article1380793654_Gaber.pdf)

<sup>11</sup> [http://www.fao.org/fishery/culturedspecies/Cyprinus\\_carpio/en](http://www.fao.org/fishery/culturedspecies/Cyprinus_carpio/en)

**Table 4 Relevant studies reported on ECHAs dissemination pages.**

Name	CAS no.	Initial weight (g)	Final weight (g)	Growth period - weeks	Feed %
<u>2,4,6-tri-tert-butylphenol</u>	732-26-3	27,7	30(?)	8	?
<u>Triethoxyoctylsilane</u>	2943-75-1	28,7	44,5-53	8	?
<u>A mixture of: triphenylthiophosphate and tertiary butylated phenyl derivatives</u>	192268-65-8	26,4	30 (?)	8	?
<u>Methylcyclohexane</u>	108-87-2	23,6	32,4	8	2% /d
<u>3-N-isoamyl-N-ethylamino-6-methyl-7-Anilino fluoran</u>	70516-41-5	23,4	30(?)	4,3	?
<u>Carbonohydrazide</u>	497-18-7	16-32	20-31	8	?
<u>1,4-bis[(2-ethyl-6-methylphenyl)amino] anthraquinone</u>	41611-76-1	20-40 (?)	21,32-29,41	4	2% /d
<u>2,2'-dimorpholinyl diethyl ether</u>	6425-39-4	21,4	35,5 - 33,05	8	2% /d
<u>tert-butyl methyl ether</u>	1634-04-4	20,2	24,3	4	?
<u>tert-butyl methyl ether</u>	1634-04-4	21,2	24	4	?

The Japanese bioaccumulation study on bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide described above is part of the training data sets of Meylan/EPIsuite, T.E.S.T. and CAESAR. You claim that predictions made with these QSAR models do not represent external predictions due to possible autocorrelation issues. None of the reported QSARs indicate that BCF for bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide will exceed 2000. You also note that information regarding the experimental data used by the software is lacking. You consider that these estimations therefore need to be regarded as less reliable. Further, for CAESAR bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide is not within the applicability domain and the results are therefore not reliable. For EPIsuite, there are conflicting results reported by you and the evaluating MSCA. Bis( $\alpha,\alpha$ -dimethylbenzyl) is estimated by you to have a BCF of 1977 according to the regression-based method (EPIsuite BCFBAF v3.01). This is in contrast to calculations made by the evaluating MSCA, where BCF is estimated to be 2301<sup>12</sup> for aquatic species. The inconsistency most likely arises as a result of the evaluating MSCA using the Log Kow value from the registration, while you use the default value for the registered substance in EPIsuite. For T.E.S.T the results are close to 2000, although the Wang and Ghose-Crippen Log Kow values (4.84 and 5.37 respectively), used as descriptors in the TEST BCF model, are both below the measured Log Kow. The consensus model in T.E.S.T. performs relatively poorly for the chemicals that are most similar to the registered substance, as is indicated by the mean absolute error (MAE in Log<sub>10</sub>) of 0.71 between predictions and experimental data. For the VEGA (KNN/Read-Across v1.1.0) model, an updated version indicates that the substance is outside of the applicability domain.

<sup>12</sup> Physical Property user-input to model based on dissemination page data:

Log Kow (octanol-water): 5.60  
 Boiling Point (deg C): 341.08  
 Melting Point (deg C): 39.80  
 Water Solubility (mg/l): 0.43

However, it is claimed that the results from the Japanese bioaccumulation study are supported by VEGA QSAR prediction (KNN/Read-Across v) of BCF= 517, as this model does not include the Japanese study in its training set. ECHA has not been able to verify the validity of the VEGA prediction, but an updated VEGA BCF model (KNN/Read-Across v1.1.0) actually does include bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide in the training set. In the updated version of the model, the substance is outside of the applicability domain because the accuracy of prediction for similar molecules found in the training set is not adequate. Also, the maximum error in prediction of similar molecules found in the training set has a high value, considering the experimental variability<sup>13</sup>. A BCF = 760 is nevertheless reported in the updated VEGA BCF model (KNN/Read-Across v1.1.0).

The QSAR estimations are collectively not considered to have an appreciable impact on the evaluation of bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide, as they are all associated with serious uncertainties.

In addition to the results described above, there is available data from two other dialkyl peroxides, di-tert-butyl 3,3,5-trimethylcyclohexylidene diperoxide (EC 229-782-3, CAS 6731-36-8, undergoing substance evaluation by the German MSCA) and [1,3(or 1,4)-phenylenebis(1-methylethylidene)] bis[tert-butyl] peroxide (EC 246-678-3, CAS 25155-25-3, undergoing substance evaluation by the Netherlands MSCA). These substances have also been tentatively<sup>14</sup> detected in aquatic and marine biota, in addition to rats from urban areas (Miljødirektoratet M-446, 2015) indicating that they may accumulate. The substances are assumed to have peroxide bonds that are shielded by tert-butyl groups, making them less likely to hydrolyze. Bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide has cumyl groups which also produce this effect ([Antonello et al 1997](#)). Tert-butyl- and cumyl-groups both yield steric hindrance, likely increasing the stability of the chemicals. Measured data also exist for di-tert-butyl 3,3,5-trimethylcyclohexylidene, showing BCF values indicating vB. However, the reliability has been ranked as low by you and subsequently disregarded due to concerns over the water solubility of the substance. It is noted that the substance evaluation decision for di-tert-butyl 3,3,5-trimethylcyclohexylidene diperoxide requests a new water solubility test to be performed.

Based on the available information it cannot be concluded that the registered substance is not bioaccumulative. The Log K<sub>ow</sub> values, unreliable BCF study and QSARs of questionable reliability indicate that a bioaccumulation study according to the updated OECD TG 305 is justified if the substance is considered persistent in the simulation study.

#### Considerations on the test method and testing strategy

Only in case the study requested under 1.1. results in the registered substance meeting the criteria for a persistent (P) or very persistent (vP) substances under REACH Annex XIII, is the new fish bioaccumulation study required.

The OECD 305 bioaccumulation test may in principle be carried out using either aqueous or dietary exposure. Bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide has a Log K<sub>ow</sub> of 5.6, while the recommendation for when to use the dietary approach is for substances with Log K<sub>ow</sub> above 5.5. Based on expert judgement, the aqueous approach may still be used taking

<sup>13</sup> (from VEGA output explanation of applicability domain)

<sup>14</sup> Term described on p14 in report referred to:

<http://www.miljødirektoratet.no/Documents/publikasjoner/M446/M446.pdf>

the solubility of the substance into account. The aqueous exposure is preferred as this will be more transparent when comparing with the REACH Annex XIII criteria for B/vB.

The following conditions in the experimental setup must be fulfilled:

- Aqueous exposure to bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide shall be used for the bioaccumulation study;
- Selection of species shall be justified by you;
- The fish shall be fed a maintenance diet. As an example, for trout this is accepted to be 2 % feed per bodyweight daily. The amount of feed shall be adjusted according to the growth of the sampled fish, i.e. by bulk weighing;
- You have the option to consider performing the OECD 305-II: Minimised Aqueous Exposure Fish Test as described in paragraphs 83–96 of the OECD 305 test guideline, if this is sufficiently justified according to the criteria in the test guideline. You also have the option to consider testing one rather than two concentrations, if this is sufficiently justified according to the criteria in paragraph 49 of the OECD 305 test guideline.

#### Alternative approaches and proportionality of the request

The request for a fish bioaccumulation test is suitable and necessary to obtain information that will allow to clarify whether the registered substance is B or vB in accordance with REACH Annex XIII. The available QSAR predictions and experimental study on fish have been evaluated and based on the available information it cannot be concluded that the registered substance is not bioaccumulative. More explicitly, there is no equally suitable alternative way available of obtaining this information. It is highlighted to you that options available in the OECD TG 305 include a minimised test, which if sufficiently justified, can be used instead of the "full" test. A further option is one rather than two concentrations. Both these options reduce the number of fish used in the study. Where the data, once obtained, confirms that the registered substance meets the PBT or vPvB criteria, it will allow authorities to consider further regulatory risk management in form of identification as a Substance of Very High Concern in accordance with REACH Article 57. ECHA notes that there is no experimental study available at this stage that will generate the necessary information without the need to test on vertebrate animals.

#### Consideration of Registrants' comments

You commented on the large variation in reported BCF values and suggest that the maximum BCF value, which is used for the lipid and growth correction, is a possible experimental outlier. While this is a possibility, ECHA points out that the value is not unique in the dataset, and that a second high value occurs in the replicate for that test concentration and time point, with BCF=1440.

Further, you argue that it would be more appropriate to use averaged uncorrected values for the calculation. While this would reduce the BCF value to a point below 2000 for the cited calculation, actual data for the lipid content and weight are still missing. The calculation performed by the evaluating MSCA was intended to demonstrate that there is a concern for BCF in a reasonable worst case scenario. Whether this actually represents the true BCF for the substance is still uncertain. Thus, there is therefore a need to perform testing on bioaccumulation, rather than performing further calculations on a poorly documented set of data.

You argue that a literature review should be performed on peroxidase activity. The

evaluating MSCA contracted the University of Oslo [REDACTED] to perform a search of the published literature to investigate this further. The conclusion, following the analysis of the existing scientific literature and websites generally available via the internet, was that no report has been found that describes an enzymatic activity able to metabolize bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide or other aryl/alkyl peroxides, neither in humans, nor in other living organisms. ECHA is aware that such claims are also made in the OECD SIDS document (2012) on Aryl Substituted Dialkyl Peroxides. These claims are not supported by citing any research literature and are therefore considered unreliable. For further information, please see literature review [REDACTED].

You cite *in vitro* data to demonstrate that there should be no concern for bioaccumulation of di-tert-butyl 3,3,5- trimethylcyclohexylidene diperoxide (EC 229-782-3, CAS 6731-36-8, undergoing substance evaluation by the German MSCA) and [1,3(or 1,4)-phenylenebis(1-methylethylidene)] bis[tert-butyl] peroxide (EC 246-678-3, CAS 25155-25-3, undergoing substance evaluation by the Netherlands MSCA). These *in vitro* data are not referenced, but the evaluating MSCA can only assume that this refers to the liver metabolism S9 fraction data on di-tert-butyl 3,3,5- trimethylcyclohexylidene diperoxide and [1,3(or 1,4)-phenylenebis(1-methylethylidene)] bis[tert-butyl] peroxide. These data are currently being considered by other evaluating MSCAs for substances under their respective substance evaluation. Regardless of the outcome of the evaluations, there will still be considerable uncertainty connected to the bioaccumulation potential of bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide until a fish bioaccumulation test has been performed. The *in vitro* metabolism rate constant is extrapolated to an overall *in vivo* metabolism rate constant on the basis of the publication by Cowan-Ellsberry et al. 2008 and this approach does not yet have an established guideline. These tests are useful weight of evidence data, but cannot alone be used to confirm or refute the bioaccumulation potential of a substance.

You consider that the OECD 305 fish test should only be performed as a last resort. It is accepted that the use of vertebrate laboratory animals should be kept to a minimum, but ECHA considers that a read-across from *in vitro* testing and literature reviews cannot be sufficient to conclude on the B concern. In order to reach a definitive conclusion on the B properties, a new fish bioaccumulation test is needed if the substance is confirmed as P or vP.

#### Consideration of Proposal(s) for Amendment and Registrants' comments to PfAs

One Member State made PfAs on the bioaccumulation test asking for more information and clarification of why the available fish bioconcentration test is inadequate to address the B concern. The reasons are now reflected in the text above. The Member State also suggested to remove the test or to include a text highlighting the possibility to perform a minimized bioaccumulation test. ECHA maintained the testing requirement, for the reasons explained above in the decision, but has provided further justification and included the suggested text on the minimized test.

#### Conclusion

Therefore, based on the substance evaluation and pursuant to Article 46(1) of the REACH Regulation, ECHA concludes that in case the study requested under request 1 results in the registered substance to meet the criteria for a persistent (P) or very persistent (vP) substances under REACH Annex XIII you are required to carry out the following study using the registered substance subject to this decision:

Bioaccumulation in fish: aqueous exposure (test method: OECD 305). The bioaccumulation or bioconcentration of the registered substance shall be assessed.

You shall revise the PBT/vPvB assessment when the new information on bioaccumulation is available.

### **References**

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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 environment (suspected PBT/vPvB) and exposure (wide dispersive use, exposure of environment, exposure of workers, consumer exposure, high RCR and high (aggregated) tonnage), bis( $\alpha,\alpha$ -dimethylbenzyl) peroxide CAS No 80-43-3 (EC No 201-279-3) 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 Norway (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.

The evaluating MSCA considered that further information was required to clarify the following concerns: suspected PBT/vPvB. 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 14 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 considered the comments received from the Registrant(s). These comments did not lead the evaluating MSCA to modify its requirements. However, some clarifications were added in 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 other Member States and ECHA for proposal(s) for amendment.

Subsequently, the evaluating MSCA received proposal(s) for amendment to the draft decision from three Member States. The ones from two of the Member States were more of editorial character and was incorporated in the Reasons (Appendix 1). The third proposed to change the testing strategy. The evaluating MSCA did not change the requests, but further data and justification is added and the PfAs 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). Your 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 did not



take into account any comments on the draft decision as they were not related to the proposal(s) for amendment made and are therefore considered outside the scope of Article 52(2) and Article 51(5).

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-53 written procedure and ECHA took the decision according to Article 51(6) of the REACH Regulation.

**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). 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 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](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 Registrant(s) to perform the stud(y/ies) on behalf of all of them.