EUROPEAN CHEMICALS AGENCY

Helsinki, 22 November 2019

Addressee: [redacted]

Decision number: CCH-D-2114488834-32-01/F
Substance name: N,N'-hexane-1,6-diylbis[3-(3,5-di-tert-butyl-4-hydroxyphenylpropionamide]
EC number: 245-442-7
CAS number: 23128-74-7
Registration number: [redacted]
Submission number: [redacted]
Submission date: 02/02/2018
Registered tonnage band: 100-1000

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (the REACH Regulation), ECHA requests you to submit information on:

1. **In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.; test method: Bacterial reverse mutation test, EU B.13/14. / OECD TG 471) with the registered substance using one of the following strains: *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102;**

2. **In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method: OECD TG 473) or in vitro micronucleus study (Annex VIII, Section 8.4.2, test method: OECD TG 487) with the registered substance;**

3. **In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: OECD TG 476 or TG 490) with the registered substance, provided that both studies requested under 1. and 2. have negative results;**

4. **Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method: Alga, growth inhibition test, EU C.3./OECD TG 201) with the registered substance**

5. **Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.; test method: Fish, early-life stage (FELS) toxicity test, OECD TG 210) with the registered substance;**

6. **Identification of degradation products (Annex IX, Section 9.2.3.; test method: Aerobic and anaerobic transformation in soil (OECD TG 307), or other appropriate and suitable test method, as further defined in the Appendix 1)**

You have to submit the requested information in an updated registration dossier by **31 May 2021**. You shall also update the chemical safety report, where relevant. The deadline has 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 and advice and further observations are provided in Appendix 3.

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, has to 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[1] by **Claudio Carlon**, Head of Unit, Hazard Assessment

[1] 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

1. In vitro gene mutation study in bacteria (Annex VII, Section 8.4.1.)

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to IX to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

According to Article 13(3) of the REACH Regulation, tests required to generate information on intrinsic properties of substances shall be conducted in accordance with the test methods recognised by the Commission or ECHA.

Other tests may be used if the conditions of Annex XI are met. More specifically, Section 1.1.2 of Annex XI provides that existing data on human health properties from experiments not carried out according to GLP or the test methods referred to in Article 13(3) may be used if the following conditions are met:

(1) Adequacy for the purpose of classification and labelling and/or risk assessment;
(2) Adequate and reliable coverage of the key parameters foreseen to be investigated in the corresponding test methods referred to in Article 13(3);
(3) Exposure duration comparable to or longer than the corresponding test methods referred to in Article 13(3) if exposure duration is a relevant parameter; and
(4) adequate and reliable documentation of the study is provided.

You provided two studies for this endpoint, a key study, non-GLP according to OECD 471, and a supporting study, pre-GLP, pre-OECD 471 (but equivalent to OECD 471), both performed in S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 strains, with and without metabolic activation and both with negative results.

According to paragraph 13 of the current OECD TG 471 test guideline (updated 1997) at least five strains of bacteria should be used: S. typhimurium TA1535; TA1537 or TA97a or TA97; TA98; TA100; S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101). This includes four strains of S. typhimurium (TA1535; TA1537 or TA97a or TA97; TA98; and TA100) that have been shown to be reliable and reproducibly responsive between laboratories. These four S. typhimurium strains have GC base pairs at the primary reversion site and it is known that they may not detect certain oxidising mutagens, cross-linking agents and hydrazines. Such substances may be detected by E.coli WP2 strains or S. typhimurium TA102 which have an AT base pair at the primary reversion site.

You have provided two tests from the year 1987 and 1978 according or equivalent to OECD TG 471, non-GLP with an assigned reliability score of 2. The tests used four different strains of S. typhimurium TA [1535, TA 1537, TA 98 and TA 100] and it did not include tests with strains S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101). However, since the test was conducted, significant changes have been made to OECD TG guideline 471 so that additionally testing with S. typhimurium TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101) is now required. Therefore, the provided study does not meet the current guidelines, nor can it be considered as providing equivalent data according to the criteria in Annex XI, 1.1.2. of the REACH Regulation.

ECHA concludes that a test using *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102 has not been submitted and that the test using one of these is required to conclude on *in vitro* gene mutation in bacteria.

In your comments on the draft decision, you agree that there is a data gap for the fifth strain in the *in vitro* gene mutation test in bacteria. Therefore, you provided a QSAR analysis using the "Ames mutagenicity S9 activated" module to address the missing fifth strain. ECHA has evaluated the provided information under the rules set in Annex XI, Section 1.3. Qualitative or quantitative structure-activity relationship (QSAR).

Annex XI, Section 1.3. states that results obtained from valid QSAR models may be used instead of testing when the cumulative conditions, as specified under Annex XI, Section 1.3., are met, one of which is that adequate and reliable documentation of the applied method is provided.

ECHA considers that based on the documentation provided, the description of the model found in the TIMES software and the scientific paper documenting the model, it is not possible to conclude whether the data provided in the training set covers the missing fifth strain of the Ames test. Furthermore, ECHA notes that on the webpage of the developer, when referring to the model only 3 strains are mentioned and not five. Consequently, ECHA does not consider the prediction reliable to address the missing fifth strain.

Therefore the adaptation you provided does not fulfil the criteria specified in Annex XI, Section 1.3. and it is rejected.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier and in your comments does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

ECHA considers that the bacterial reverse mutation test (test method EU B.13/14. / OECD TG 471) is appropriate to address the standard information requirement of Annex VII, Section 8.4.1. of the REACH Regulation.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Bacterial reverse mutation test (test method: EU B.13/14. / OECD TG 471) using one of the following strains: E. coli WP2 uvrA, or E. coli WP2 uvrA (pKM101), or S. typhimurium TA102.

2. In vitro cytogenicity study in mammalian cells or in vitro micronucleus study (Annex VIII, Section 8.4.2.)

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to IX to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

An "*In vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study" is a standard information requirement as laid down in Annex VIII, Section 8.4.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

While you have not explicitly claimed an adaptation, you have provided information that could be interpreted as an attempt to adapt the information requirement according to Annex VIII, Section 8.4.2., column 2:

"*an in vitro cytogenicity study in mammalian cells or in vitro micronucleus study does not need to be conducted because adequate data from an in vivo cytogenicity test are available*" However, ECHA notes that your adaptation does not meet the specific rules for adaptation of Annex VIII, Section 8.4.2., column 2 because of the reasons explained below.

For this endpoint the registrant provided three in vivo studies, an in vivo micronucleus study, a sister chromatide exchange study and an in vivo dominat lethal test.

In the in vivo micronucleus study equivalent or similar to OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test), non GLP (1982), there are several deficiences which make the study questionable:

- The samples seems to have been collected 24 hrs after exposure which may be too late. The bone marrow samples should have been collected between 18-24hrs. As stated in the test guideline, this required harvest time of between 18-24hrs is a consequence of the kinetics of appearance and disappearance of the micronuclei in this tissue compartment.
- At least 2000 immature erythrocytes per are animal are requsted by the TG OECD 474. In the provided study only 1000 cells per animal were assessed.
- Furthermore, the provide table doesn't show the PCE/NCE (polychromatic/normchromatic cells) ratio which would provide information on whether the bone marrow has been reached.

Therefore, the validity of this test cannot be confirmed.

In the sister chromatide exchange study equivalent or similar to EPA OTS 798.5915 (In Vivo Sister Chromatid Exchange Assay), non GLP (1981), only 4 animals were used instead of 5 and it is stated in the dossier that only chromatides of 2 animals per group and sex were examined. Furthermore, testing guideline states that "*Animals should be treated with test chemical followed by administration of BrdU. BrdU may be administered by multiple IP injections, by continuous tail vein infusion or by subcutaneous implantation of tablets*." However, in the dossier a different procedure was applied: "*Two hrs before application of test or control substance each animal is applied with 5 -bromodeoxyuridine (BUdR)* "

The thymidine analogue 5-bromo-2′-deoxyuridine (BrdU) has been widely used to make sister chromatid differentiation (SCD) evident in metaphase chromosomes of cells grown for two cycles in BrdU and, thus, containing varying amounts of the thymidine analogue. If BrdU is added after cells were treated with a DNA-damaging agent, the effect on SCEs can only be analyzed in the second post-treatment mitosis. However, no details on this aspect are given in the dossier. Finally, the sister chromatide exchange test provides only an indication of induced damage to DNA but not direct evidence of mutation nor can replace the requirement for a cytogencity test.

In the in vivo dominat lethal test equivalent or similar to OECD Guideline 478 (Genetic Toxicology: Rodent Dominant Lethal Test), non-GLP (1978), using a single dosing, only 6 mating periods covering all stages of germ cell maturation from the A-spermatogenia to the spermatozon were included. The TG OECD 478 states that: "*For a single treatment up to five daily dose administrations, there should be 8 (mouse) or 10 (rat) matings conducted at weekly intervals following the last treatment.*" and that " *All treatment and mating schedules should be scientifically justified.*" Furthermore, while it is generally accepted that dominant lethals are due to structural and numerical chromosome aberrations this study investigate whether chemicals produce mutations resulting from chromosomal aberrations in the germ cells. Therefore the cytogenicity potential in somatic cells cannot be elucidated.

Consequently, the provided in vivo studies are insufficient to cover the information requirement for this endpoint.

Therefore, your adaptation of the information requirement is rejected.

In your comments on the draft decision, you have provided information that could be interpreted as an attempt to adapt the information requirement according to Annex XI, Section 1.2. Weight of evidence (WoE) of REACH.

Annex XI, Section 1.2 states that there may be sufficient weight of evidence (WoE) from several independent sources of information leading to assumption/conclusion that a substance has or has not a particular dangerous (hazardous) property, while information from a single source alone is insufficient to support this notion.

The sources of information submitted as part of the WoE must be adequate and reliable to enable a conclusion on whether the registered substance has or has not a hazardous property for chromosomal aberrations in mammalian cells.

To support your WoE approach you indicate the following:

1. None of the available *in vivo* studies in the dossier show concern for genotoxicity,
2. The QSAR analysis (also provided in your comments) using the "Chromosomal Aberrations S9 activated" module gives a negative prediction for both the parent compound and all potential metabolites; and
3. other chemicals of this substance class have shown negative results in various genotoxicity tests.

As already explained above, ECHA notes that the information provided for the *in vivo* studies is not adequate and reliable.

As regards the QSAR analysis ECHA notes that the prediction for the *in vitro* cytogenicity in the QMRF files, Sections 4.2 Explicit algorithm, and sections 4.3-4-6, related to molecular descriptors, are unsatisfactorily described. ECHA furthermore notes that information on internal validation and external validation is not documented. The applicability domain is specified in broad ranges for calculated parameters like molecular weight and log Kow but the distribution of chemical within ranges is not discussed. Without the algorithm being clear and transparent, it is not possible to judge what statistics is provided in the QMRF. Therefore, ECHA concludes that the QSAR model and the resulting prediction, is not sufficiently described. Based on available and missing information, and on the fact that chromosome aberration relates to DNA binding, as well as to protein binding (and 5th strain in Ames test is missing), and observed inconsistencies in documentation, ECHA considers that the prediction for *in vitro* mammalian chromosomal aberration cannot be considered valid.

Finally, with reference to point 3 above, ECHA notes that in the absence of information on which substances and what tests you are referring to, ECHA cannot assess the validity of this argument.

In view of the above, from the sources of information you provided it is not possible to evaluate the possible hazardous property for chromosomal aberrations in mammalian cells.

Based on the assessment above, it is therefore not possible to conclude, based on any source of information alone or considered together, and taking into account your justification for the weight of evidence adaptation whether the registered substance has or has not the particular

dangerous hazardous property foreseen to be investigated in an OECD TG 473 or OECD TG 487 study. Your adaptation is rejected and the information requirement is not fulfilled.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier and in your comments does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

ECHA considers that the *in vitro* mammalian chromosome aberration test (test method OECD TG 473) and the *in vitro* mammalian cell micronucleus test (OECD TG 487) are appropriate to address the standard information requirement of Annex VIII, Section 8.4.2. of the REACH Regulation.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: *In vitro* mammalian chromosome aberration test (test method: OECD TG 473) or *in vitro* mammalian cell micronucleus study (test method: OECD TG 487).

3. In vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.)

In accordance with Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at 100 to 1000 tonnes per year must contain, as a minimum, the information specified in Annexes VII to IX to the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

An "*In vitro* gene mutation study in mammalian cells" is an information requirement as laid down in Annex VIII, Section 8.4.3. of the REACH Regulation, "if a negative result in Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2." is obtained.

You have sought to adapt this information requirement according to Annex VIII, Section 8.4.3., column 2. You provided the following justification for the adaptation:

"*In accordance with Annex VIII (8.4.3) of the REACh legislation, an in vitro gene mutation study does not need to be conducted if adequate data from a reliable in vivo gene mutation assay is available. In this case, the test substance was found to be non carcinogenic in two valid studies performed with rats.*"

However, ECHA notes that your adaptation does not meet the specific rules for adaptation of Annex VIII, Section 8.4.2., column 2 because of the reasons explained below.

The *in vivo* data provided by you to adapt this endpoint are two old carcinogencity studies. Although carcinogenicty studies can provide useful information on genotoxicity, ECHA notes that the available studies do not constitute adequate data from a reliable *in vivo* gene mutation assay for the following reasons:

The first one, a key study from 1980, is a pre-GLP Combined Chronic Toxicity/Carcinogenicity (OECD 453) rat feeding study with the registered substance using 70 animals/sex/dose. Four doses were used (0, 500, 1500 and 5000 ppm) meaning that in total 560 rats must have been used. However, you stated that "*Three hundred and fifty rats (185 males and 165 females) died or were killed in extremis during the treatment period*" due to "*non-treatment-related reasons*". The testing guideline for OECD 453 states that "*Each dose group (as outlined in paragraph 22) and concurrent control group intended for*

the chronic toxicity phase of the study should contain at least 10 animals of each sex, in the case of rodents" and that "*For rodents, each dose group (as outlined in paragraph 22) and concurrent control group intended for the carcinogenicity phase of the study should therefore contain at least 50 animals of each sex.*" In addition, the animals used in the chronic toxicity phase of the study, normally of 12 months duration, provide interim kill data for the carcinogenicity phase of the study, thus achieving a further reduction in the number of animals used overall.

From the data presented in the dossier the number of animals left for statstical analysis in the end of the study is likely to have been very low and the significance of data interpretation questionable.

The second carcinogencity study is a supporting, non-GLP, non guideline 2-year rat feeding study from 1979 with only 20 males and 20 females in the dose and control groups. The TG OECD 451 (Carcinogenicity Studies) states that "*A sufficient number of animals should be used so that a thorough biological and statistical evaluation is possible. Each dose group and concurrent control group should therefore contain at least 50 animals of each sex.*". One male and three females from dose groups (which group was not reported) and 5 animals in the concurent control died during the experiment (no further details). The study concluded that the test item did not induce neoplastic changes in Wistar rats of both sexes treated during 2 years via the diet at a dose level of 100 mg/kg diet. However, due to the low number of animals used, the statistical significance of the findings is questionable. Furthermore, the purity of the tested substance is not reported and no verification of doses or concentrations was performed. Therefore, due to the deficiencies listed above this study does not provide sufficient evidence to conclude that there is no hazard potential for *in vivo* gene mutation.

Therefore, your adaptation of the information requirement is rejected.

In your comments on the draft decision you again indicated that this information requirement can be waived due to the available negative carcinogenicity studies.

Firstly, as already explained above, ECHA notes that the carcinogenicity and the *in vitro* gene mutation in mammalian cells endpoints are separate information requirements under REACH. According to REACH Annex VIII, section 8.4.3., the *in vitro* gene mutation study in mammalian cells is required if there is a negative result in 8.4.1. and 8.4.2. Furthermore, according to Annex VIII, section 8.4.3., column 2, an *in vitro* gene mutation study in mammalian cells does not need to be conducted if adequate data from a reliable *in vivo* mammalian gene mutation test are available. ECHA notes that adequate data from a reliable *in vivo* mammalian gene mutation test are not available in your technical dossier.

Secondly, in your comments you refer to OECD guidance document 116 stating that "*After 18 months, the lowest survival observed was 74.29 % in the female control group, all other dose groups showed even higher survival after 18 months. Similarly, after 24 months the lowest survival rate observed was 30% (male animals dose group 3), all other survival rates were higher. The number of surviving animals were therefore always above the threshold specified in the OECD guidance document 116 that termination should be considered if survivors fall below 25%".* ECHA however notes that the paragraph 162. of OECD GD 116 states: *"For a negative result to be acceptable in a rat carcinogenicity bioassay, survival in the study should ideally be no less than 50% in all groups at 24 months, while for "life span studies", studies continued to end of life/ death of the animals survival at study termination should not be less than 25%".* ECHA notes that the survival rates for male and female animals are less than 50% in all groups at 24 months in the

study you provided. Therefore, the significance of the data interpretation remains questionable and the negative result is not acceptable.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier and in your comments does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

ECHA considers that the *in vitro* mammalian cell gene mutation tests using the *Hprt* and *xprt* genes (OECD TG 476) and the *in vitro* mammalian cell gene mutation tests using the thymidine kinase gene (OECD TG 490) are appropriate to address the standard information requirement of Annex VIII, Section 8.4.3.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: *In vitro* mammalian cell gene mutation test (test method: OECD TG 476 *or* OECD TG 490) provided that both studies requested under 1. and 2. have negative results.

4. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.)

"Growth inhibition study aquatic plants" is a standard information requirement as laid down in Annex VII, Section 9.1.2. of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

In the technical dossier you have provided a study record for a key study (reference title: Report on the growth inhibition test to Green Algae (Scenedesmus subspicatus)"). However, this study does not provide the information required by Annex VII, Section 9.1.2., because it is not reliable due to the following reasons.

In the study submitted the algae were exposed to nominal test concentrations of *"0, 1.23, 3.77, 11, 33 and 100 mg/L under static conditions"* with no analytical determination of test concentrations. As results of the study you indicate that *"Clear effects were observed at 100 mg/L test substance"* and report that *"NOEbC and EbC50 values based on cell number were 11 and 45 mg/L, respectively and clearly above the limit of water solubility"*. While effects were observed on the tested species you conclude that the tested substance is *"with high probability acutely not harmful to aquatic algae and cyanobacteria" as "no toxic effects could be recorded in the range of water solubilty"*.

Your substance is difficult to test due to low water solubility, and in the study a vehicle was used to obtain test concentrations above the water solubility limit. No analytical monitoring took place and the effects are expressed based on nominal concentratiosn alone. The OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, ENV/JM/MONO (2000)6/REV1 (8 Feb 2019) provides the opportunity to use nominal concentrations to define effects when it is not possible to analytically quantify the concentration causing effects. In your dossier, you have not provided any reasoning as to why analytical monitoring was not possible in the study. Due to lack of analytical monitoring and nominal concentrations in excess of water solubility, it is not possible to know at what concentration the effects were observed.

Additionally, you derived the (nominal) effect concentration based on biomass. As laid down in ECHA Guidance on information requirements and chemical safety assessment, Chapter

R.7b (version 4.0, June 2017) while both acute growth rate EC50 (ErC50) and biomass (EbC50) endpoints are reported the EbC50 should not be used since direct use of the biomass concentration without logarithmic transformation cannot be applied to an analysis of results from a system in exponential growth. If the effect value can only be reported based on biomass a new study should be considered to be performed.

In your comments on the Proposal for Amendment (PfA), based on which this request was added to the decision, you indicate that the study is valid since the validity criteria given in OECD TG 201 (paragraph 11) have been fulfilled, as clear dose dependent effects were observed and since you have been able to, based on reevalution of the raw data, to calculate an effect value based on growth rate.

ECHA acknowledges that the validity criteria regarding the growth of the control cultures have been fulfilled. In your comment, you indicate that a clear dose response was observed. However as discussed above, due to lack of analytical monitoring and nominal concentrations in excess of water solubility, it is not possible to know at what concentration the effects were observed. In the endpoint study record (ESR) it is stated that "*small parts of the test substance were swimming on the surface of the test water at test concentrations of 11, 33 and 100 mg/L*", which further highlights the unclarity of exposure concentrations. Furthermore, according to ECHA Guidance on information requirements and chemical safety assessment Chapter R.7b (Version 4.0, June 2017) (p. 80) studies where undissolved test material is present and effects are observed should be considered invalid.

Consequently, there is an information gap and it is necessary to provide information for this endpoint.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: a growth inhibition study on aquatic plants.

In the study analytical monitoring of the test solutions should be performed. If it is not technically feasible to conduct chemical analysis of the test solution, it should be demonstrated that all reasonable analytical efforts were attempted.

Notes for your consideration

Due to the low solubility of the substance in water you should consult OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, ENV/JM/MONO (2000)6/REV1 (8 Feb 2019) and ECHA Guidance on information requirements and chemical safety assessment (version 4.0, June 2017), Chapter R7b, Table R.7.8-3 summarising aquatic toxicity testing of difficult substances for choosing the design of the requested ecotoxicity test(s) and for calculation and expression of the result of the test(s).

5. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1.)

"Long-term toxicity testing on fish" is a standard information requirement as laid down in Annex IX, Section 9.1.6. of the REACH Regulation. Adequate information on Fish, early-life stage (FELS) toxicity test (Annex IX, 9.1.6.1.), or Fish, short-term toxicity test on embryo and sac-fry stages (Annex IX, 9.1.6.2.), or Fish, juvenile growth test (Annex IX, 9.1.6.3.) needs to be present in the technical dossier for the registered substance to meet this information requirement.

You have sought to adapt this information requirement according to Annex IX, Section 9.1.6., column 2. You provided the following justification for the adaptation: *"In Annex IX of Regulation (EC) No 1907/2006, it is laid down that a study on long-term toxicity to fish shall be proposed by the registrant if the chemical safety assessment indicates the need to investigate further the effects on fish. According to Annex I of this regulation, the chemical safety assessment triggers further action when the substance or the preparation meets the criteria for classification as dangerous according to Directive 67/548/EEC or Directive 1999/45/EC or is assessed to be a PBT or vPvB. The hazard assessment of the substance reveals neither a need to classify the substance as dangerous to the environment, nor is it a PBT or vPvB substance, nor are there any further indications that the substance may be hazardous to the environment. Therefore, and for reasons of animal welfare, a long-term toxicity study in fish is not provided."*

However, ECHA notes that your adaptation does not meet the specific rules for adaptation of Annex IX, Section 9.1.6., column 2 due to the following.

ECHA Guidance on information requirements and chemical safety assessment Chapter R.7b (Version 4.0, June 2017) explains in section R.7.8.4.3 "Exposure considerations for aquatic pelagic toxicity requirements" the context of this Annex IX, Section 9.1.6., column 2 adaptation rule. According to the Guidance, the need to conduct further (long-term) testing is indicated for example when due to low water solubility of a substance, short term toxicity tests do not reveal any toxicity. In such cases long-term testing is required to appropriately assess the potential risk of the substance to the environment.

ECHA notes that the registered substance is poorly water soluble (WS < 0.01 mg/l). ECHA Guidance on information requirements and chemical safety assessment Chapter R.7b (Version 4.0, June 2017) further explains why short-term tests may not give a true measure of toxicity for poorly soluble substances. Poorly water soluble substances require longer time to be significantly taken up by the test organisms and, consequently, the duration of short-term toxicity test is likely to be insufficient to reach steady state conditions. For this reason, short-term tests may not give a true measure of toxicity for poorly soluble substances. Accordingly, long-term toxicity cannot be excluded and should be investigated.

The available acute aquatic toxicity tests (on invertebrates and fish) on the registered substance reveal no effects up to the limit of water solubility of the registered substance. Therefore it is not possible to determine the relative sensitivity of the species. As a consequence, the Integrated testing strategy (ITS) outlined in ECHA Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017) (Section R.7.8.5 including Figure R.7.8-4), is not applicable in this case.

Lastly, for the environmantal hazard assessment (Annex I, section 3.0 of REACH), the available toxicity information should at least cover species of three trophic levels: algae/aquatic plants, invertebrates (Daphnia preferred), and fish. As explained above, for poorly soluble substance only long-term studies can be used to fully assess the risks to the aquatic environment the long-term data on the missing trophic level, fish, is also required.

In your comments on the Proposal for Amendment (PfA), based on which this request was added to the decision, you indicate that no long-term fish study is needed due to no acute and chronic effects observed in the available aquatic studies. However, as discussed above acute data is meaningless due to the low solubility of the registered substance. Furthermore, effects were observed in the OECD TG 201 study even if the nature and level of effects for aquatic plants is currently unknown and needs to be further studied (request

4.). As also fully discussed above, long-term data on three trophic levels, including fish, is required to fully assess the risks to the aquatic environment.

In your comments on the PfA you also note that no study is needed due to the substance having a low potential for bioaccumulation. However, substance's potential to bioaccumulate is not an acceptable adaptation for the current endpoint. Furthermore, there are separate standard information requirements for bioaccumulation and long-term toxicity to fish in REACH as these studies have different scopes and assess different properties of a substance, its potential to accumulate in organisms and its potential to cause long-term toxicity. Low bioaccumulation can also not be used to demonstrate low exposure of aquatic organisms as a substance may cause toxicity on the long-term even at low body concentrations Therefore, ECHA considers that the information currently available does not rule out potential for long-term risk to the environment and there is a need to investigate further the effects on aquatic organisms.

Therefore, your adaptation of the information requirement cannot be accepted.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirement. Consequently there is an information gap and it is necessary to provide information for this endpoint.

According to ECHA *Guidance on information requirements and chemical safety assessment, Chapter R.7b* (version 4.0, June 2017) fish early-life stage (FELS) toxicity test (test method OECD TG 210), fish short-term toxicity test on embryo and sac-fry stages (test method EU C.15. / OECD TG 212) and fish juvenile growth test (test method EU C.14. / OECD TG 215) can be performed to cover the standard information requirement of Annex IX, Section 9.1.6.

However, the FELS toxicity test according to OECD TG 210 is more sensitive than the fish, short-term toxicity test on embryo and sac-fry stages (test method EU C.15 / OECD TG 212), or the fish, juvenile growth test (test method EU C.14. / OECD TG 215), as it covers several life stages of the fish from the newly fertilized egg, through hatch to early stages of growth (see ECHA *Guidance on information requirements and chemical safety assessment* (version 4.0, June 2017), *Chapter R7b, Section R.7.8.4.1.*

Moreover, the FELS toxicity test is preferable for examining the potential toxic effects of substances which are expected to cause effects over a longer exposure period, or which require a longer exposure period of time to reach steady state (ECHA *Guidance Chapter R7b,* version 4.0, June 2017).

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Fish, early-life stage (FELS) toxicity test (test method: OECD TG 210).

Notes for your consideration

Once results of the test on long-term toxicity to fish are available, you shall revise the chemical safety assessment as necessary according to Annex I of the REACH Regulation.

Due to the low solubility of the substance in water you should consult OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures, ENV/JM/MONO (2000)6/REV1 (6 July 2018) and ECHA *Guidance on information requirements and chemical safety assessment* (version 4.0, June 2017), Chapter R7b, Table R.7.8-3 summarising aquatic toxicity testing of difficult substances for choosing the design of the requested

ecotoxicity test(s) and for calculation and expression of the result of the test(s).

6. Identification of degradation products (Annex IX, Section 9.2.3.)

The identification of the degradation products is a standard information requirement according to column 1, Section 9.2.3. of Annex IX of the REACH Regulation. Adequate information on this endpoint needs to be present in the technical dossier for the registered substance to meet this information requirement.

In the technical dossier you have provided some information on potential degradation products. You have indicated that according to CATALOGIC 301C (v.09.13) prediction submitted under the endpoint of ready biodegradation (IUCLID section 5.2.1) 3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionic acid (CAS 20170-32-5, EC 243-556-1) is the main metabolite of the registered substance. In the QPRF of the CATALOGIC prediction, a number of other potential metabolities has been identified by their structure and SMILES codes alone.

However, this information does not provide the information required by Annex IX, Section 9.2.3., because of the following.

Based on the information available, the metabolities have been identified by the CATALOGIC model alone. While, according to the QPRF provided, the registered substance fits the general parametric and structural domain of this model, the transformation reliability was low for most of these metabolites. The low reliabilities (between 0.01 to 0.37) indicate that in the Catalogic 301C these transformations are not well supported by available biodegradation data. Hence, it is unclear what metabolites would be formed in quantities >=0.1% and at what rate they would be formed. Furthermore, the substance's low water solubility and potential for microbial toxicity flagged by the model further hamper the reliability of the prediction of the metabolities.

In your comments on the Proposal for Amendment (PfA), based on which this request was added to the decision, you indicate that the CATALOGIC prediction is valid as the substance falls within the applicability domain of the model. As given above ECHA agrees that the substance fulfils the parametric domain of the model, including the range of water solubility as its lower threshold in the model is zero. Nevertheless, the low solubility of the registered substance affects the reliability of the prediction and makes it questionable whether the transformation products would be formed in the predicted quantities in the context of a 28 days MITI study set up (OECD 301C) used in the prediction. Regarding the metabolic domain, ECHA notes that as given above the transformation reliability is low (between 0.01 to 0.37). Hence, even if the substance is within the applicability domain, meaning that it has been recognised and matched by the training set of the model, some transformation reactions are not well supported by available biodegradation data. The prediction is hence of low reliability. As discussed in more detail below it is necessary to have reliable information on the degradation products formed, and in particular on whether they are formed under relevant conditions.

The information on predicted transformation/degradation products are hence not adequate for the purpose of risk assessment, and hence does not fulfil the requirements for acceptance of QSARs set in Annex XI, section 1.3, of the REACH Regulation.

According to Annex IX, Section 9.2.3., column 2 of the REACH Regulation, identification of degradation products is not needed if the substance is readily biodegradable. ECHA notes

that based on the information in the technical dossier, the registered substance is not readily biodegradable (OECD TG 301B up to 5 % degradation in 28 days).

Furthermore, ECHA considers that information on transformation and/or degradation products is needed in relation to the PBT/vPvB assessment and risk assessment that also need to cover its relevant transformation and/or degradation products.

As explained above, the information provided on this endpoint for the registered substance in the technical dossier does not meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

Regarding the appropriate and suitable test conditions and methods, as the substance has a water solubility of < 10 µg/l, and is also highly adsorptive (log Koc = 6.5-8.9), adsorption to soil and sediment is likely. Therefore, soil and sediment simulation test (OECD TG 307 and TG 308) can be considered as appropriate test methods to study degradation of the registered substance. Based on the uses reported in the technical dossier, soil exposure cannot be excluded [redacted]
The aerobic and anaerobic transformation in soil (test method: OECD TG 307) is therefore the preferred test to cover this endpoint and to obtain information on degradation products. Due to the high adsorption potential of the registered substance formation of Non Extractable Residues might occur. Therefore in your test results you should explain and scientifically justify the extraction procedure and solvent used obtaining a quantitative measure of NER.

In the test each relevant transformation/degradation product shall be assessed. This can be done simultaneously during the same study. Assessment of relevant degradation/transformation products is described in ECHA Guidance on information requirements and chemical safety assessment (version 3.0, June 2017), Chapter R.11 PBT/vPvB assessment.

You may also use other appropriate and suitable test methods to provide information on the degradation products for example by enhanced screening level degradation test or modelling tools. In any case, the provided information should include, identification, stability, behaviour, molar quantity of metabolites relative to the parent compound. In addition, degradation half-life, log Kow and potential toxicity of the metabolites may be investigated. You will need to provide a scientifically valid justification for the chosen method.

Providing accurate information on the transformation and/or degradation products of the registered substance is particularly important since the main metabolite identified by you is in ECHA's Annex III inventory identified as likely to meet criteria for category 1A or 1B carcinogenicity, mutagenicity or reproductive toxicity and may hence fulfil the T-criterion of Annex XII of REACH. Nevertheless it is necessary to emphasise that the present information requirement of identification of degradation products is not yet adequately fulfilled and it is unknown whether the main and other relevant degradation products are formed in relevant conditions.

In section 2.3 of your IUCLID dossier (PBT assessment) you have indicated that the possible main transformation/degradation product(s) do not qualify as bioaccumulative. You also indicate this in your comments on the PfA. However, ECHA considers this information as not yet sufficient to conclude the PBT/vPvB assessment of the substance and/or its

degradation/transformation products since as discussed above the information provided on transformation/degradation products is not yet sufficient to fulfil the present standard information requirement. If it is shown that this suspected degradation product is formed during the study, also its bioaccumulation potential as that of any other relevant transformation/degradation products formed, would need to be fully assessed.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision:

Identification of the degradation products (Annex IX, Section 9.2.3.) OECD TG 307, or other appropriate and suitable test method, as described above. ECHA recommends to use OECD TG 307, as specified above.

Deadline to submit the requested information in this decision

The timeline indicated in the draft decision to provide the information requested was 12 months from the date of adoption of the decision. Following the receipt of proposals for amendment from the competent authority of a Member State, requests for information on Growth inhibition study aquatic plants (Annex VII, Section 9.1.2), Long-term toxicity testing on fish (Annex IX, Section 9.1.6.1) and Identification of degradation products (Annex IX, Section 9.2.3) were added to the decision. As a consequence, the timeline was amended to 18 months from the date of the adoption of the decision.

Appendix 2: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of your registration after the date when the draft decision was notified to you under Article 50(1) of the REACH Regulation.

The compliance check was initiated on 24 May 2018.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

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

ECHA took into account your comments and did not amend the requests.

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

ECHA received proposals for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendments.

ECHA referred the draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

The Member State Committee reached a unanimous agreement on the draft decision in its MSC-66 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 compliance check decision does not prevent ECHA from initiating further compliance checks on the present registration at a later stage.

2. Failure to comply with the requests in this decision will result in a notification to the enforcement authorities of your Member State.

3. In relation to the information required by the present decision, the sample of the substance used for the new tests must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants.

   It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new tests is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured or imported by each registrant.

   If the registration of the substance by any registrant covers different grades, the sample used for the new tests must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grades registered to enable the relevance of the tests to be assessed.